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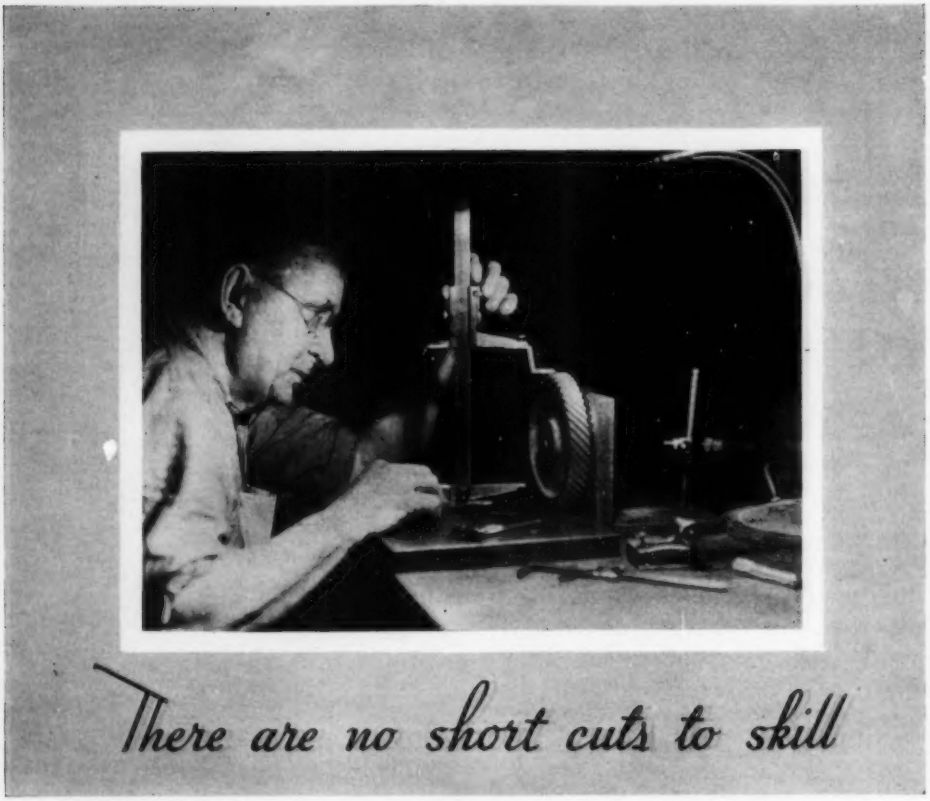

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JOURNAL OF DAIRY SCIENCE

VOLUME XX JUNE, 1937 NUMBER 6

THE LACTOGENIC PREPARATIONS FROM THE ANTERIOR PITUITARY AND THE INCREASE OF MILK YIELD IN COWS

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INTRODUCTION

After the well-known papers of Grüter and Stricker (11, 20), showing the possibility of influencing the lactation of farm animals by extracts from the anterior pituitary, few papers have subsequently appeared confirming these preliminary observations (8, 15, 16, 19) on the rôle of the anterior pituitary in the lactation phenomena. In 1932, Riddle, Bathes and Dykshorn (17) discovered the hormone of lactation, "prolactin." This discovery has stimulated still more the study of physiology of the hypophysis and of its rôle in the lactation of Mammalia.

Since January, 1932, my collaborators and I have carried out a series of experiments on laboratory animals (rabbits, guinea-pigs, dogs, rats) and on cows, investigating the mechanism of lactation generally and of the increase of milk yield in cows. In a series of tests we are able to prove, first of all, that the so-called prolan (from the urine of pregnant women) is not capable of inducing lactation (in dogs, rabbits) or of stimulating milk yield in cows (1, 2). In this respect our results disagree with the findings of Hupka and Majert (12), according to whom prolan seems to influence the increase of milk yield in cows.

We also did considerable work in the production and study of different lactogenic preparations from the anterior pituitary. Some of these preparations produced in the form of powder are similar in their action to liquid lactogenic preparations from the anterior pituitary (3). The purpose of this work was to secure the production of a lactogenic preparation free from other hormones of the anterior pituitary (gonadotropic and thyreotropic hormones, growth hormone, etc.). Besides, we have naturally tested the lactogenic preparations produced by the method of Riddle, Bathes and

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Dykshorn (17), as well as by the method of Lyons and Catchpole (14) and of Turner and Gardner (10).

Laboratory animals (rabbits, etc.) were used as controls and also cows and pigs on which were tried absolutely all variations of the preparations. It has to be noted that from the very beginning a sharp difference became apparent between the action of two groups of preparations upon the course of lactation in the cow: preparations of the type of prolactin of Riddle, Bathes and Dykshorn and lactogenic preparations produced mainly by the method of Evans (9), *i.e.*, preparations containing besides prolactin several other hormones of the hypophysis. Preparations of the prolactin type, while giving invariably a positive reaction on pigeons and rabbits, did not induce in our experience an increase of the milk yield in lactating cows, both normal and castrated. In the cows a positive effect could always be produced by the so-called *total* preparation from the anterior pituitary, *i.e.*, by the lactogenic preparation from the anterior pituitary containing a whole number of the hypophysis hormones. This statement holds good even when the doses of "pure" prolactin were increased two, three and more times compared with the "total" preparations. Even if the "pure" prolactin sometimes gave a positive result (increased milk yield) it was very unsteady and that only after doses ten times and higher than those of the "total" preparation.

We have already mentioned that by the name of "total" preparation we have in our experiments conditionally called the extract from the anterior pituitary produced by us, with some slight modifications, by the method used by Evans to produce the growth hormone (9, 3). This preparation was later improved by us, produced in a dry state, freed considerably by repeated freezing from superfluous proteins, etc. We usually used freshly prepared lactogenic preparations, although we also secured positive results from preparations (dry) stored for six and more months.[1]

During the described period (since 1932) we have carried out numerous tests on a large number of cows (in all considerably over 2000 head of different breeds, age, number of lactations, month of lactation, under different conditions of feeding, at different seasons, barren and pregnant, normal and

[1] *Method of preparation.* The anterior pituitary of cattle is thoroughly ground and diluted with six times its volume of distilled water, temperature +1° C., stirred for 30 minutes and then added 1.5 volumes of 0.2 N $Ba(OH)_2$, also previously cooled. Mixing continued for one hour and then placed overnight on ice, temperature 0° to +4° C. The extract is separated from the remains of tissue by filtration through gauze. To the extract is added 0.2 N H_2SO_4 till obtaining about pH 8.0. Ten minutes later a few crystals of Na_2SO_4 are added. The extract is placed on ice over night, then centrifuged and the liquid conserved with a few drops of tricresol. Keep on ice. In order to obtain a frozen preparation the centrifuged extract is frozen at −10° C. After thawing an almost limpid liquid is poured off, which is used for injecting the cows. The "dry" preparation was obtained by precipitating the alkaline extract at the isoelectric point by adding 1% solution of acetic acid. The precipitate is dried with non-aqueous acetone. Before use this powder is diluted in 1% solution of Na_2HPO_4. The insoluble residue is thrown out.

castrated. It is obvious that we have gathered much very valuable experimental material, part of which was printed by us in Soviet publications (1, 2, 3, 4, 5, 6, 18). The sum total of our tests rendered it possible to arrive at present at a definite conclusion, that namely, *the lactogenic preparations from the anterior pituitary made in our laboratory induce an increase of the milk yield in lactating cows. In certain series of tests this increase of the milk yield has reached seven and more liters of milk per day (three milkings).*

This increase of the milk yield takes place not only upon *a single* injection of the preparation, but also when it is used repeatedly in a prolonged experiment (18) in which the total preparation had been injected every ten days to a group of cows during 3½ months, *i.e.*, each cow received 10 injections. A fresh extract from the anterior pituitary was prepared each time. All cows, with few exceptions, have reacted by an increase of the milk yield after each injection of the preparation. Thus by this test (and by other tests), it was shown that it is possible to repeat the injection of the lactogenic substances from the hypophysis not only once but several times and to get in each case an increase of the milk yield.

As we have gradually gathered considerable practical material of several series of tests in each of which however participated only a relatively small group of animals it became logically necessary: (a) to arrange a simultaneous experiment on a large herd of cattle at one State Farm; (b) to check finally the possibility of *practical* application of the lactogenic preparations of the hypophysis.

This was accomplished in November, 1935, to January, 1936, at one of the large State Farms in the vicinity of Moscow.

SELECTION OF ANIMALS

Originally it was intended to carry out the test at one cowbarn only (the 4th) on 170 head of cattle, but, as the injection of the preparation to 138 cows at cowbarn No. 4 gave during 12 days more than 1900 liters of milk above the usual production, we decided at the request of the management of the State Farm to include three more cow barns (2nd, 3rd and 5th).

Thus in the experiment were included milk cows at four cowbarns of the State Farm with the exception of: (a) champion cows, (b) dry cows, (c) cows within 20 days after calving and (d) obviously sick cows. In all, injections were given to 510 cows. A control group of 90 cows was selected as well (Table 1).

TABLE 1

Distribution of cows in the cowbarns

GROUP/COWBARNS	NO. 4	NO. 2	NO. 3	NO. 5	TOTAL
Experimental	138	115	154	103	510
Control	28	18	22	22	90
Total	166	133	176	125	600

Breed. The bulk of experimental animals consisted of non-pedigree cows, 220 head (36.6 per cent of the total number—600 cows), and Yaroslav, 186 head (31 per cent), followed by Kholmogor, 65 head (10.8 per cent), Simmental, 67 head (11.2 per cent). The rest was made up by a small number of such breeds as Swiss (4.2 per cent), Red Gorbatov, Red German, Tagil, etc. (Table 2).

TABLE 2

Breed of test animals

GROUP	NON-PEDIGREE	YAROSLAV	KHOLMOGOR	SIMMENTAL	SWISS	RED GORBATOV	RED GERMAN	TAGIL	BROWN LATVIAN	DANISH	YUTLAND	TOTAL
Experimental	182	164	53	56	21	17	2	6	3	5	1	510
Control	38	22	12	11	4	1	–	–	1	1	1	90
Total	220	186	65	67	25	18	2	6	4	6	1	600

Age. Table 3 shows that the bulk of experimental animals—421 cows (71.2 per cent)—consisted of cows born between 1924 and 1928, *i.e.*, cows with 7–10 calvings.

TABLE 3

Age of test animals

GROUP	YEAR OF BIRTH														TOTAL
	1920 and earlier	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	
Experimental	10	9	35	48	97	76	95	62	30	13	10	6	17	2	510
Control	.....	1	7	13	16	17	16	4	7	1	3	...	4	1	90
Total	10	10	42	61	113	93	111	66	37	14	13	6	21	3	600

Milk yield. The milk yield of cows in about 50 per cent of cases amounted to 6 to 12 l. Table 4 gives an idea of the average daily milk yield during the preliminary period.

TABLE 4

Distribution of cows according to milk yield (preliminary period)

COWBARN NUMBER	MILK YIELD IN LITERS (FROM—TO)								
	1–3	4–6	7–9	10–12	13–15	16–18	19–21	22–24	25–27
No. 4	.....	9.3	19.6	30.4	19.6	12.1	3.5	.....	.....
No. 2	3.5	9.5	27.8	22.6	19.1	12.1	3.5	1.8	.....
No. 3	1.3	16.9	24.0	24.4	18.1	9.0	2.5	1.3	2.5
No. 5	.....	7.7	23.0	30.0	12.5	17.3	9.6	.....	.....

Note.—The figures represent the percentage of cows yielding a given amount of milk.

TABLE 5

Distribution of cows according to live weight

GROUPS	LIVE WEIGHT OF TEST AND CONTROL ANIMALS (KG.)															
	300–325	326–350	351–375	376–400	401–425	426–450	451–475	476–500	501–525	526–550	551–575	576–600	601–625	626–650	WEIGHT UNKNOWN	TOTAL
Experimental	1	5	12	39	58	95	66	88	61	42	11	5	7	4	16	510
Control	...	...	3	5	6	17	10	17	9	14	3	4	...	1	1	90
Grand total	1	5	15	44	64	112	76	105	70	56	14	9	7	5	17	600

TABLE 6

State of pregnancy

COWBARN NO.	GROUPS	BARREN				PREGNANT				GRAND TOTAL
		Recently calved	From 4 to 11 months	Over 11 months	Total	1 to 4 months	5 to 7 months	Total	Month unknown	
4	Experimental	28	48	8	84	27	25	52	2	138
	Control	5	6	6	17	5	6	11	...	28
	Total	33	54	14	101	32	31	63	2	166
2	Experimental	24	31	.....	55	28	30	58	2	115
	Control	4	4	1	9	3	5	8	1	18
	Total	28	35	1	64	31	35	66	3	133
3	Experimental	28	43	7	84	38	30	68	2	154
	Control	7	7	2	16	.....	6	6	...	22
	Total	35	50	9	100	38	36	74	2	176
5	Experimental	9	20	1	30	47	25	72	1	103
	Control	2	3	1	6	9	7	16	...	22
	Total	11	23	2	36	56	32	88	1	125
Altogether in the test	Experimental	89	148	16	253	140	110	250	7	510
	Control	18	20	10	48	17	24	41	1	90
	Total	107	168	26	301	157	134	291	8	600

TABLE 7

Distribution of the animals according to the month of lactation

COW-BARN	GROUPS	MONTH OF LACTATION																				UN-KNOWN	TOTAL
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
4	Experimental	9	14	10	7	12	21	21	12	8	6	3	6	1	2	...	...	...	1	...	1	...	3	138
	Control	2	4	1	1	3	3	3	3	2	3	...	1	...	1	...	...	...	1	...	...	...	.....	28
	Total	11	18	11	8	15	24	24	15	10	9	3	7	1	3	...	...	...	2	...	1	...	3	166
2	Experimental	.....	13	12	8	12	7	12	15	14	7	6	2	2	...	1	...	1	...	...	3	...	1	115
	Control	.....	1	4	.....	1	1	2	2	2	1	2	...	...	2	...	...	...	...	...	...	...	.....	18
	Total	.....	14	16	8	13	8	14	17	16	8	8	2	2	2	1	...	1	...	...	3	...	1	133
3	Experimental	.....	12	18	11	10	13	16	16	13	6	11	2	5	5	3	...	...	2	1	1	1	8	154
	Control	.....	3	4	1	1	3	2	2	1	2	...	...	1	1	...	...	...	...	...	...	...	.....	22
	Total	.....	15	22	12	11	16	18	18	14	8	11	2	6	6	3	...	...	2	1	1	1	8	176
5	Experimental	.....	6	8	0	5	15	22	12	8	6	6	1	1	...	...	1	...	2	...	...	0	1	103
	Control	.....	2	1	2	2	2	3	2	3	.....	2	1	...	...	...	...	...	1	...	1	...	1	22
	Total	.....	8	9	11	7	17	25	14	11	6	8	2	1	...	...	1	...	3	...	1	...	2	125
	Altogether in the test:																							
	Experimental	9	45	48	35	39	56	71	55	43	25	26	11	9	7	4	1	1	5	1	5	1	13	510
	Control	2	10	10	4	7	9	10	9	8	6	4	2	1	4	...	...	...	2	...	1	0	1	90
	Grand total	11	55	58	39	46	65	81	64	51	31	30	13	10	11	4	1	1	7	1	6	1	14	600

Live weight. The bulk of the animals was composed of cows weighing from 425 to 500 kg. (Table 5). Taking into account the breed it can be stated that the cows were in good or above the average state of fleshiness. Varied rations rich in protein contributed to it.

Pregnancy. All the experimental animals can be divided into 4 groups: (a) barren after recent delivery (1–3 months after calving), (b) barren (during 4–11 months), (c) barren (over 11 months), and (d) pregnant cows. As can be seen from Table 6 in our experiment were included 301 barren cows (50.1 per cent of the total).

Lactation month. Table 7 shows that in 85 per cent of cases the animals had a normal course of lactation (from 1 to 10 months).

Control group. In the control group were gathered animals (Tables 2–7) approaching in all indices as near as possible the cows, which received injections of the preparation from the anterior pituitary.

FEEDING AND KEEPING

The cows were fed at cowbarns individually twice a day and milked 3 times a day. Cows at cowbarn No. 4 were receiving the following ration: steppe hay composed of different herbs (7–8 kg.), dried sugar-beet press (1–4 kg.) and a mixture of concentrates, comprising 40 per cent of sunflower oil cake, 30 per cent of group oats and 30 per cent of wheat bran (1.5–10 kg.). One kg. of this mixture equals 0.99 feed unit and contains 153 grams of protein. With each meal the cows also received mineral food made up of common salt and ground chalk.

The ration of the other cowbarns included: meadow hay of medium quality, sugar beet press, mixture of concentrates consisting of 40 per cent of sunflower oil cake, 30 per cent of barley, as well as mineral food. In addition at cowbarns No. 2 and 5 sunflower silage was given to the amount of 50 per cent of the total quantity of succulent feeds.

The cows received as much water as they wanted from the automatic waterers.

After injection of the anterior pituitary preparation the milk yield increased in the great majority of cows. At that period the cows received additional concentrates, usually following the increase in the milk yield.

However at different cowbarns different variations of additional feed were tried. The following method was adopted at cowbarn No. 4. From the beginning of the preliminary period (11/28/1935) up to the end of the test all experimental animals (reserved for injection as well as control ones) were placed on surplus ration, receiving a surplus of concentrates calculated on the basis of additional two liters of milk above the actual average daily milk yield. Beginning with the second day of the increase in the milk yield (under the influence of the preparation) all cows received additional concentrates calculated on the basis of actual increase in the milk

yield the day before. In proportion to the gradual drop in the milk yield following the ceasing of the action of the lactogenic preparation the additional feed was also reduced.

At the other three cowbarns we used a different system of additional feeding. Remaining before injection on the usual farm ration, experimental as well as control animals began to receive during the period of the action of the preparation, starting with the second day of the increase in the milk yield, additional concentrates. This additional feed, which corresponded to the maximum increase of the milk yield, lasted until the end of the experiment, when in spite of the continued additional feeding, the milk yield started to fall following the cessation of the action of the preparation until it had reached the former pre-injection level. However in many cows the increase in the milk yield lasted throughout the experimental period. The control group of cows also received supplementary concentrates calculated on the basis of minimum two additional liters of milk per day per cow. As will be seen later it nevertheless had little effect on the increase of milk yield on the control group.

INJECTIONS OF THE PREPARATION AND REGISTRATION OF THE MILK YIELD

In the present experiment we used the total preparation from the anterior pituitary of cattle. Injections were given as usual, subcutaneously, in the region of the neck, each injection consisting of 50 cc. of freshly made up preparation, which corresponds approximately to about six grams of the hypophysis. There was not a single case of painful affection following the injection of the preparation.

At the fourth cowbarn the measuring of the milk yield was done by weighing on special weighing machines and the amount of milk was then expressed in liters. At the other cowbarns the measuring was done by a milk-meter.

RESULTS OF THE EXPERIMENT

As usual in our experiments, the increase of the milk yield in cows receiving injections started in about 24 hours and lasted several days. At the fourth cowbarn two injections were given with 15 days' interval. The increase of the milk yield at the fourth cowbarn after both injections, as well as at the other barns lasted from 6 to 15 days.

Such irregularity is undoubtedly connected with individual properties of the cows, as well as with the feeding conditions in those cowbarns. The latter seems to be the main reason if we remember the conditions of additional feed at the fourth cowbarn.

Figure 1 shows the fluctuations of the milk yield at the fourth cowbarn 9 days before the injections and the milk yield after the first and second injections.

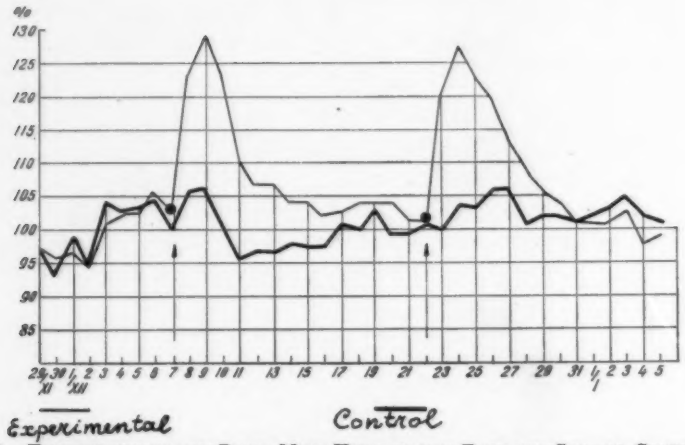

FIG. 1. FLUCTUATION OF THE DAILY MILK YIELD OF THE TEST AND CONTROL GROUPS OF COWS AT COWBARN NO. 4 (EXPRESSED AS PERCENTAGE). THE AVERAGE MILK YIELD DURING THE PRELIMINARY PERIOD IS TAKEN AS 100 PER CENT.

If the average milk yield at this cowbarn 9 days before the injections amounted to 1508 l. per day (within the limits 1417–1594 l.) the daily increase of the milk yield in days following the first injection amounted to 350 l., 440 l., 168 l., 101.5 l., 105 l. and then less (Table 8). Expressed as a percentage, the milk yield after injection amounted to (if the average daily milk yield at the cowbarn before the injection is taken at 100 per cent) 123.2 per cent, 129 per cent, 122.5 per cent, 111.1 per cent, 106.8 per cent, etc. The daily milk yield at that cowbarn before injection varied from 93.9 to 105.7 per cent. We see a similar picture at the fourth cowbarn after the second injection as well (table 8).

TABLE 8

Daily increase of milk yield after injection (in liters)

COW-BARN NO.	AV. DAILY MILK YIELD PER COWBARN (PRELIM. PERIOD)	INJECTION	DAYS											TOTAL
			1	2	3	4	5	6	7	8	9	10		
4	1508	1st.	350	440	340	168	101	105	65	62	37	45	253 11–15th day	3872
		2nd	307	419	344	284	196	118	82	59	13	21	63 11–12th day	.....
2	1210	.....	274	293	233	145	66	60	52	.....	.....	.....	.....	1123
3	1567	.....	226	377	300	183	181	148	65	28	54	.....	.....	1562
5	1240	.....	249	266	242	193	102	76	.....	.....	.....	.....	.....	1118
In all	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7675

The fluctuations in the milk yield of the control group were insignificant. The total quantity of additional milk produced at the fourth cowbarn (by 138 cows) amounted to 3871 l.

The same trend is observed at the other cowbarns (Figs. 2, 3 and 4, Table 8). Expressed as percentage, the daily increase of the milk yield at the second cowbarn in cows receiving injections of the preparation amounted

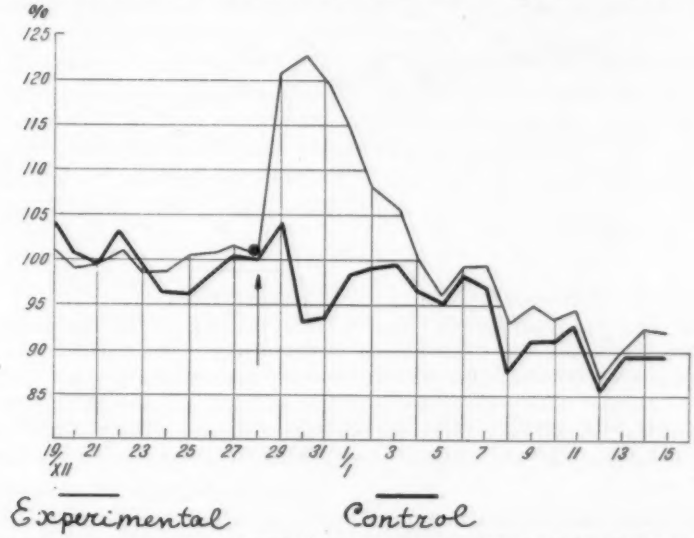

FIG. 2. FLUCTUATION OF THE DAILY MILK YIELD OF THE TEST AND CONTROL GROUPS OF COWS AT COWBARN NO. 2 (EXPRESSED AS PERCENTAGE). THE AVERAGE MILK YIELD DURING THE PRELIMINARY PERIOD IS TAKEN AS 100 PER CENT.

to 122.6 per cent, 124.2 per cent, 119.3 per cent, 112.0 per cent, 105.4 per cent, and less; the fluctuations in the milk yield of the control group did not rise above 101.0 per cent (here also the average daily milk yield for the preliminary period was taken as 100 per cent). The total additional quantity of milk produced by giving injections of the preparation at the second cowbarn (by 115 cows) amounted to 1123 l.

At the third cowbarn the milk yield after injections was as follows: 114.4 per cent, 124 per cent, 119.1 per cent, 111.6 per cent, 111.5 per cent, 109.3 per cent, 103.9 per cent, etc. Before the injections the milk yield in this group of cows did not rise above 101.8 per cent (as compared with the average daily milk yield taken as 100 per cent). The milk yield in the control group of cows did not rise during the test period above 102.5 per cent. Total quantity of additional milk at the third cowbarn (from 154 cows)—was 1562 l.

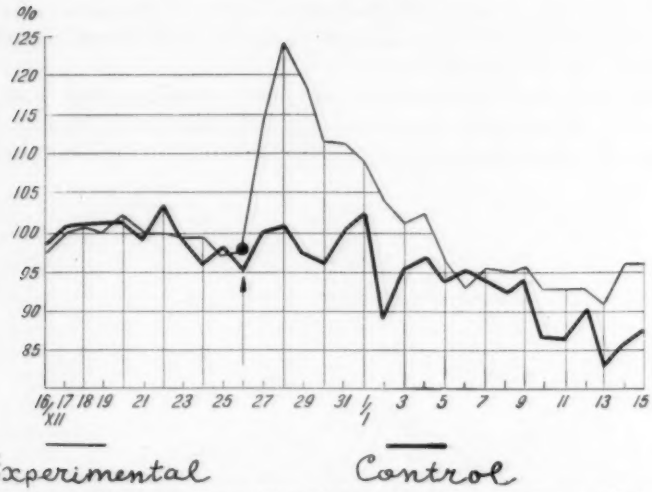

FIG. 3. FLUCTUATION OF THE DAILY MILK YIELD OF THE TEST AND CONTROL GROUPS OF COWS AT COWBARN NO. 3 (EXPRESSED AS PERCENTAGE). THE AVERAGE MILK YIELD DURING THE PRELIMINARY PERIOD IS TAKEN AS 100 PER CENT.

At the fifth cowbarn after injection the cows of the test group gave 120.1 per cent, 121.5 per cent, 119.5 per cent, 114.8 per cent, 108.2 per cent,

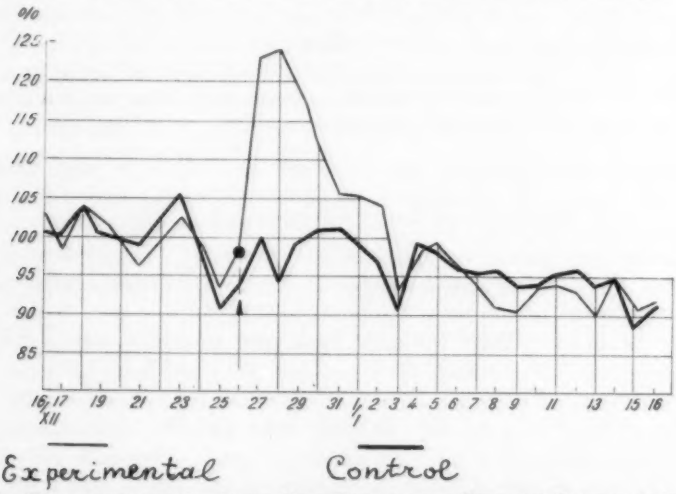

FIG. 4. FLUCTUATION OF THE DAILY MILK YIELD OF THE TEST AND CONTROL GROUPS OF COWS AT COWBARN NO. 5 (EXPRESSED AS PERCENTAGE). THE AVERAGE MILK YIELD DURING THE PRELIMINARY PERIOD IS TAKEN AS 100 PER CENT.

106.0 per cent (compared with the preliminary period). Total quantity of additional milk at the fifth cowbarn (from 103 cows)—1118 l.

As a result of the injections of the preparation from the anterior pituitary 510 cows gave 7675 l. of additional milk above the quantity which those cows would have produced without the injections.

The above-mentioned data show clearly that a notable increase in the milk yield was obtained only from cows which were given injections. The control group produced only a negligible quantity of additional milk. The data also make it clear that the additional quantity of milk is produced in the main not as a result of additional feeding. Two facts support this contention: (1) In spite of continued additional feeding the cows cease to give an increased milk yield after the injected preparation has ceased to act; (2) In spite of received additional feed the control group of cows did not increase the milk yield to any marked extent.

DISCUSSION

First of all it is necessary to confront the following data. Each cow at cowbarn No. 4 gave on the average 14 l. of additional milk per one injection, at cowbarns No. 2, No. 3, and No. 5—9.8, 10.0, and 10.9 liters, respectively. The better results at cowbarn No. 4 can probably be explained by the better feeding conditions at that cowbarn compared with the other barns. This gives a certain reason for the conclusion that better keeping of cows favorably affects the results from injections of the lactogenic substances from the anterior pituitary.

We could confirm it also by a test carried out at another State Farm.

Individual cows respond differently to the injection of the preparation. Some cows do not react at all. At the fourth cowbarn there were four such cows, at the second 12, at the third 13, and at the fifth cowbarn four cows.

The duration of the increase of the milk yield varies with different cows. The condition at cowbarn No. 4 was as follows: After the first injection the increase lasted in 16.5 per cent of cows for 15 days, in 9 per cent for 7 days, in 11 per cent for 6 days, in 9.8 per cent for 3 days, etc.

After the second injection the increase lasted in 21.8 per cent of cows for 14 days, in 8.9 per cent for 12 days, in 10 per cent for 10 days, in 16.7 per cent for 8 days, in 9 per cent for 6 days. in 13 per cent for 5 days, in 9 per cent for 4 days, etc.

Thus at cowbarn No. 4 we had a considerable percentage of cows responding during a long period of time to the injection of the preparation from the anterior pituitary. About the same occurred at the other cowbarn with the exception that the action of the preparation only in exceptional cases lasted 14 or 15 days and was limited mostly from 7 to 10 days.

A considerable number of cows have responded to the injection of the preparation by a marked increase in the milk yield. Thus 75 per cent of

TABLE 9

Maximum increase of the daily milk yield

INCREASE OF THE YIELD (%)	IN THE FOLLOWING PERCENTAGE OF COWS			
	Cowbarn No. 4	Cowbarn No. 2	Cowbarn No. 3	Cowbarn No. 5
0	2.9	10.4	8.4	3.9
From 1 to 10	.....	0.9	.....	0.9
" 11 " 20	6.5	16.5	15.6	18.1
" 21 " 30	21.8	27.8	36.4	30.5
" 31 " 40	34.0	20.0	17.0	32.4
" 41 " 50	19.6	15.6	12.3	9.5
" 51 " 60	6.6	5.2	3.9	2.9
" 61 " 70	4.3	0.9	3.9	0.9
" 71 " 80	1.5	1.8	1.9	0.9
" 81 " 90	0.7	.....	.....	.....
" 91 " 100	2.1	0.9	.....	.....

experimental cows at the fourth cowbarn after each injection gave a 20 to 50 per cent increase in the milk yield. We speak naturally of the maximum increase in the daily milk yield (Table 9). The same is observed as at the other cowbarns. At the second cowbarn such an increase was given by 66 per cent of cows and at the 5th cowbarn by 72 per cent of cows. We see here again the same relation which has been already recorded by us: the better the feeding conditions are at the cowbarn (in our case the fourth), the greater percentage of cows responds by a larger increase of the milk yield. However at all the four cowbarns, as it is shown in Table 9, more than half of the cows responded to the injection of the preparation by a considerable increase of the daily milk yield. A certain number of cows increased the milk yield even more than by 50 per cent. A more vivid picture of the increase of the milk yield is given by Table 10.

We see here that at all the four cowbarns (but especially at the fourth) the milk yield of several cows has increased by four and more liters a day with three milkings.

Some cows are in this respect of exceptional interest. Cow No. 1172, Yaroslav, born in 1925, second month of pregnancy and fifth month of

TABLE 10

Cows with a large increase of the milk yield 4–5 liters

NUMBER OF COWBARN	DAILY INCREASE OF MILK YIELD					
	4–5 liters		5–6 liters		6–7 liters	
	Number of cows	Per cent	Number of cows	Per cent	Number of cows	Per cent
4	50	35.6	13	9.4	5	3.7
2	21	18.3	6	5.2	2	1.7
3	23	14.9	2	1.3	...	.....
5	13	12.6	3	2.9	1	0.9

lactation produced an average daily milk yield during nine days preliminary period of 13 l. On the first day after the first injection she gave 18 l., on the second day 19 l.; after the second injection 19.5 l. and 19.5 l. She maintained increased milk yield 15 days after the first injection (gave during that time 40.5 l. additional milk) and 14 days after the second injection gave during that time 57 l. additional milk. In all during 29 days cow No. 1172 gave 97.5 l. additional milk, with an average daily increase of 3.36 l. Cow No. 850, Yaroslav, born in 1924, 6th month of lactation, barren, produced an average daily milk yield during preliminary period of 10 l. On the first day after the first injection she gave 15 l., on the second day 14 l.; after the second injection she gave 15.5 and 14.5 l. She maintained increased milk yield after first injection for 15 days (36.5 l. of additional milk during that time); after the second injection for 14 days she gave 48 l. additional milk. In all during 29 days cow No. 850 gave 59 l. additional milk.

Cow No. 724, Kholmogor, born in 1928, fifth month of lactation and 3rd month of pregnancy (calved normally in June, 1936) produced an average daily milk yield during preliminary period of 11 l. After the first injection she gave daily 16 and 16.5 l.; after the second injection 14.5 and 15.5 l. She maintained increased milk yield after the first injection for 13 days (gave during that period additional 28 l.; after second injection for 14 days (gave

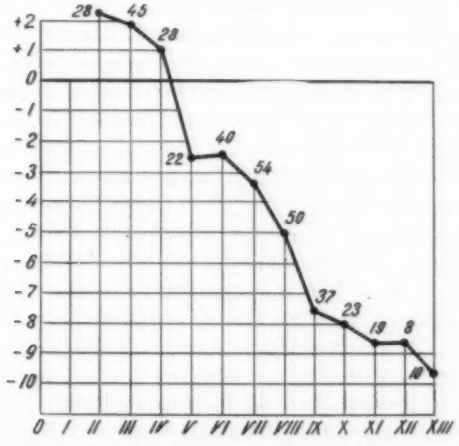

FIG. 5. AVERAGE DIFFERENCE BETWEEN THE MAXIMUM DAILY MILK YIELD AFTER CALVING AND AFTER INJECTIONS OF THE PREPARATION. ABOVE THE LINE IS SHOWN THE DIFFERENCE IN MILK YIELD, EXCEEDING THE MAXIMUM MILK YIELD OF COWS AFTER CALVING AS COMPARED WITH MILK YIELD AFTER INJECTIONS. FIGURES ON THE CURVE REPRESENT THE NUMBER OF COWS. ABSICCA REPRESENTS MONTHS OF LACTATION.

additional 27.5 l.). In all during 27 days cow No. 724 gave 55 l. additional milk.

The following fact is of special interest. We have computed average values for the increase of the milk yield of 364 cows, combining together animals from different cowbarns according to the months of lactation. It appears that the later in the period of lactation the injection is made the less is the effect of increased milk yield. Thus, if the injection during the second to fourth month of lactation can induce a rise in the milk yield exceeding the maximum daily milk yield of the cow after calving, an injection of the preparation given in the later months of lactation no longer produces the same effect (Fig. 5). This fact is recorded by us not for the first time and was already explained in the sense that during the earlier months of lactation the mammary gland seems to be more "labile" and easier increases its secretory activity under the stimulating influence of the lactogenic substances from the hypophysis.

Leaving for the next communication a detailed analysis of the reasons why individual cows react differently to the injection of lactogenic preparation let us dwell in conclusion on the following questions directly connected with our experiment.

1. Some time ago we examined in a special test the quality of milk produced after the injection of lactogenic preparations. Milk sugar, chlorine, acidity, chloro-sugar coefficient were quite normal. The percentage of butterfat in several cases was slightly increased. In the present experiment we have examined only the butterfat. In 50 cows (45 experimental and 5 control) at the fourth cowbarn the butterfat was examined in the course of 6 days before the injection of lactogenic preparation and during 5 days after injection; fat determination was repeated at that barn after second injection as well. It was found that the percentage of butterfat has risen after first injection in 12 cows and after second injection in 14 cows by 0.1 to 0.7 per cent (and on some days by 0.8 per cent).

However this rise of the fat percentage does not last long (2–3 days). It is also necessary to point out, that the butterfat percentage has increased only in cows receiving *non-frozen* total preparation from the anterior pituitary. This observation served as an incentive to detailed study of this question in which we are at present engaged.

2. Already before this test we have shown that our preparation is absolutely free from any harmful influence upon: (a) the course of lactation; (b) the development of the fetus; (c) the calving (there was not a single case of abortion attributable to the influence of lactogenic preparations); (d) the weight of new-born calves; (e) the next lactation; (f) the live-weight[2] of cows, etc. This refers also to cases of repeated injections as well.

[2] Our observations show that in many cows the live weight even increases a little.

Thus observations of the results of injecting lactogenic preparations from the anterior pituitary have shown us that in spite of the stimulation of lactation and a considerable increase of the milk yield in some cows under the influence of lactogenic substances, the subsequent behavior of cows is normal in all respects. The injection of lactogenic preparations not only does not cause subsequent depression of lactation or "exhaustion" of the productive capacity of the cow, but, as it has been shown by observations, even retards in several cases a little longer the natural drop of the lactation curve.

3. Our experiments, and especially those described in the present communication (experiments of the application of lactogenic preparations on a larger scale), have shown that the maximum effect from those substances is produced under conditions of good feed regimen. But the use of lactogenic substances under such conditions is more effective because cows better nourished and kept on plentiful rations respond better to lactogenic stimuli than cattle kept under less favorable conditions. It follows that the injection of lactogenic preparations of the hypophysis may become one of practical measures applied at dairy farms.

The practical application of the lactogenic preparations from the anterior pituitary is in general more profitable on a well-run farm than on a farm with a poor food basis or where cattle are kept under unsatisfactory conditions.

4. In the summer of 1936 we carried out important experiments (on several hundreds of cattle) at a State Farm and at several Collective Dairy Farms in the Moscow region. On the whole this large scale experiment has produced results analogous to the results described in the present article. However it is necessary to point out that the use of lactogenic substances is slightly less effective in summer than in winter. After the change to stall-feeding the effect from the use of lactogenic substances rises again.

5. Preliminary calculations have shown that the cost of an injection of lactogenic substances from the hypophysis is very low. In any case the experiment on 600 cows described in the present communication (including 90 control animals, which also received additional concentrated feeds) brought a net profit of several thousand rubles after deducting all expenses.

The injection of lactogenic substances from the hypophysis serves our laboratory as one of the methods of studying the physiology of lactation in general. The question remains open—what mechanism increases the milk yield under the influence of lactogenic preparations from the hypophysis? Does the lactogenic preparation change the secretory activity of the mammary glands, does it influence the regulation of that process, or is, which is more probable, the lactogenic stimulus connected with both? All those are questions awaiting to be settled. At present we are studying those problems.

Thus the stimulation of lactation in the cow by the use of lactogenic preparations from the anterior pituitary is not simply getting from cows additional quantities of milk, but also a profound study of one of the still unrevealed physiological phenomena—lactation.

SUMMARY AND CONCLUSIONS

1. The experiment carried out at a State Farm on 600 head of cattle, including 90 control animals, has shown that the injection of lactogenic preparation (total preparation from the anterior pituitary) can produce a real and considerable although temporary increase of the milk yield.

2. A single injection of the lactogenic preparation to 372 cows and repeated injections to 138 cows resulted in 7675 liters of additional milk in a couple of days. The quality of milk remains normal. The percentage of butterfat slightly increases in a certain number of cases.

3. The tests with the lactogenic preparation of the hypophysis have proved the absolute harmlessness of those injections to the cow's organism and the absence of any undesirable after effects upon the productivity of the animals.

4. The injection of lactogenic substance from the hypophysis is more effective in well-kept cattle.

5. The injection of the lactogenic substances is more effective during the first half of lactation (2–6 months).

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OBSERVATIONS ON THE SALTING OF BRICK CHEESE

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The manufacture of Brick cheese is not as standardized a procedure as is the manufacture of some of the other well known types of cheese. The process is described by Thom and Fisk (1), Sammis (2), and Wilson and Price (3), but there appear to be differences of opinion among these writers concerning the methods of manufacture. Differences are even more noticeable when one observes factory processes. One of the most variable procedures in commercial practice is the operation of salting.

Brick cheese is commonly salted by rubbing dry salt on the outside of the cheese once each day for two or three consecutive days or by placing the cheese in a brine bath for a day or more. There is little uniformity in salting treatments among factories, either in respect to the time of salting, duration of salting, strength of brine or amount of salt, if dry salt is used. It is generally recognized that these treatments must influence the quality of the cheese although there is considerable confusion concerning the specific effects. It is the purpose of this paper to discuss the effects of some common variations in salting treatments.

Jackson and Morris (4) have investigated the effects of some variations in concentrations of brine for salting Brick cheese. They used concentrations ranging from 10 to 25% sodium chloride and pasteurized the solutions frequently to prevent bacterial spoilage. They concluded that brines containing less than 15% salt or more than 25% were not suitable; that the moisture content of the cheese decreased as the strength of the brine increased; and that a cooking temperature of 108° F. (42.2° C.) followed by salting for 48 hours in an 18 to 22% brine produced the most desirable quality.

Fleishman (5) in discussing the brine salting of Backsteinkäsen pointed out the necessity of clean brine tanks, careful regulation of salt concentration and temperature, and uniform exposure to the brine of all surfaces of the cheese. He indicated that dry salting tended to cause greater losses of moisture and required more time and labor than brine salting and also tended to affect unfavorably the uniformity of quality.

Mrozek (6) noticed when Limburger cheese was salted in 15% brine that there was a movement of salt into the cheese and that moisture was lost. Immediately after salting, the outer layers of the cheese were rich in salt and low in moisture. After one week, the salt content of the inner and outer layers were practically the same, but the moisture in the outer layers

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was lower than at the center. Decomposition of the cheese during ripening caused marked hydration of the protein in the outer layers.

Koestler (7) called attention to the fact that when cheese was placed in a solution of sodium chloride containing more than 16% salt there was a loss of moisture from the outer portions of the cheese. This movement of moisture was reversed and an actual swelling or intake of moisture resulted when the cheese was placed in salt solutions of concentrations less than 16%.

Riddet, Valentine, McDowall and Whelan (8) studied the effect of salt on the quality of Cheddar cheese. They found that salting procedures must be varied to conform to the physical properties of the curd such as moisture content, acidity and rate of acid development. Under-salting resulted in the production of a cheese which was pasty and weak in body, which was open in texture and which did not ripen normally. Over-salting gave a cheese which was harsh in body, which ripened very slowly, and though the main portion of the cheese tended to be close in texture, the cheese rind often was badly cracked.

EXPERIMENTAL METHODS

Manufacturing Procedures

The manufacturing process used in these experiments followed most closely that described by Wilson and Price (3). A low-acid development was obtained by using only 0.1% of starter consisting of a mixture of half *S. lactis* and half *S. thermophilus* milk-grown cultures. Metal hoops instead of the usual wooden ones were used for sanitary reasons. Variations in the salting treatments will be described in connection with each experiment.

Temperatures and humidities maintained during salting and curing were somewhat low. The cheese was salted at 60° F. (15.6° C.). During the first two weeks of curing this same temperature was maintained with a humidity of 85%. The cheese was rubbed and washed every other day with a weak salt solution made by dissolving a handful of salt in a 12 quart pail of cold water. The cheese was paraffined at 14 days of age and placed in a curing cellar at 40° F. (4.4° C.) and at a relative humidity of 75%.

ANALYSES

Acidity measurements and tests for moisture and salt were made at definite intervals throughout the curd-making and ripening processes. The cheese was sampled by cutting the bricks in two at the center and removing a cross-section slice approximately 5″ x 3″ x $\frac{3}{8}$″. The rind, to a depth of one-eighth of an inch was always discarded. The remainder of the slice was used for all analyses. Two more samples for analysis could be taken from the same brick by cutting each remaining half of the brick in two and removing slices in the same manner. When it was necessary to study the

rate of salt penetration, the usual slice of cheese was divided into four layers. The outermost layer of the rind was cut away to a depth of one-eighth of an inch and discarded. The next layer five-sixteenth inches in thickness was then removed and designated the "Outer" layer. A "Middle" layer was obtained by removing another layer of the same thickness. The remainder of the slice formed the "Center" portion. Deter-

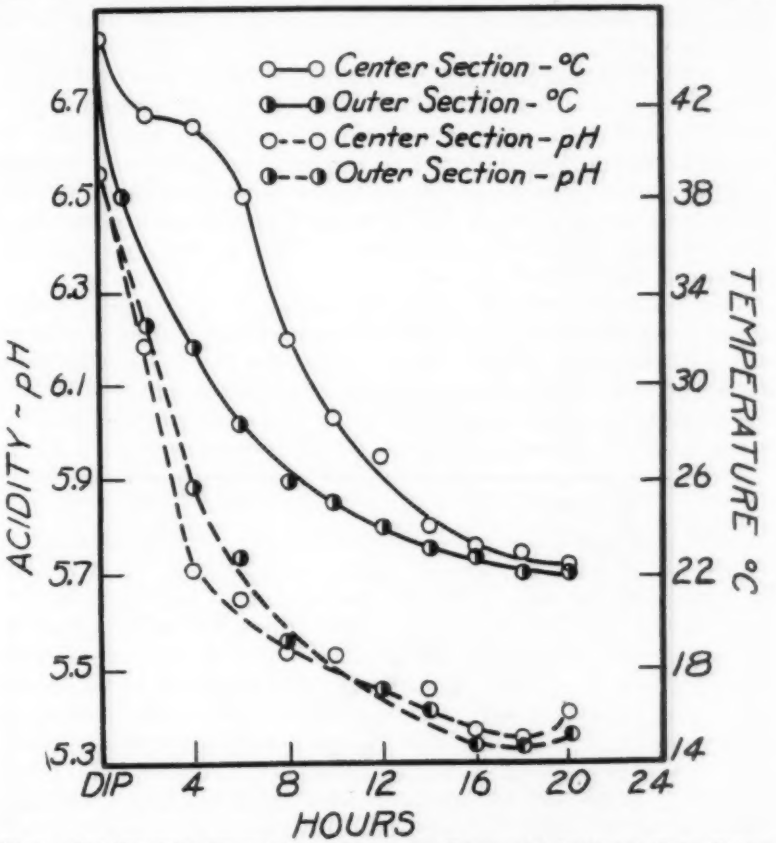

FIG. 1. THE RELATION BETWEEN TEMPERATURE AND CHANGES IN ACIDITY IN BRICK CHEESE DURING THE OPERATION OF DRAINING.

minations of pH were made with a Leeds-Northrup portable potentiometer, using the quinhydrone electrode. The amount of salt present was determined by titration according to the method described by McDowall and Whelan (9). Moisture tests were made by weighing about four grams of cheese in aluminum cups and drying at 100° C. for 24 hours at atmospheric

pressure, followed by one hour under a vacuum of at least 20 inches. The samples were then cooled in a desiccator and reweighed.

RESULTS

Observations on Changes in Cheese During Making and Salting

Eight lots of cheese were used to study changes in acid, moisture and salt during curd-making and curing. Milk selected from the same herd of cows was used for all eight lots. In this manner it was possible to follow a more uniform making process from day to day. Two of these eight lots are of special interest because measurements were made on one of them at intervals of 2 hours for a period of 20 hours after dipping; while similar observations were made on the second lot at intervals of 12 hours for 68 hours after dipping and then at intervals of 24 hours or more for 220 hours. The observations on the other six lots of cheese approximated so closely those which occurred in these two special lots that only these two lots will be used to illustrate the trends of early changes.

The composition of curd during the making of Brick cheese is determined by the interaction of a number of forces but chiefly those of temperature, acid and rennet effects. Figure 1 illustrates changes in temperature and acidity in the outer and center portions of a single loaf of cheese. While the temperature remained above 32 to 34° C. the acid development was rapid. Acid development decreased at temperatures below 30° C. and

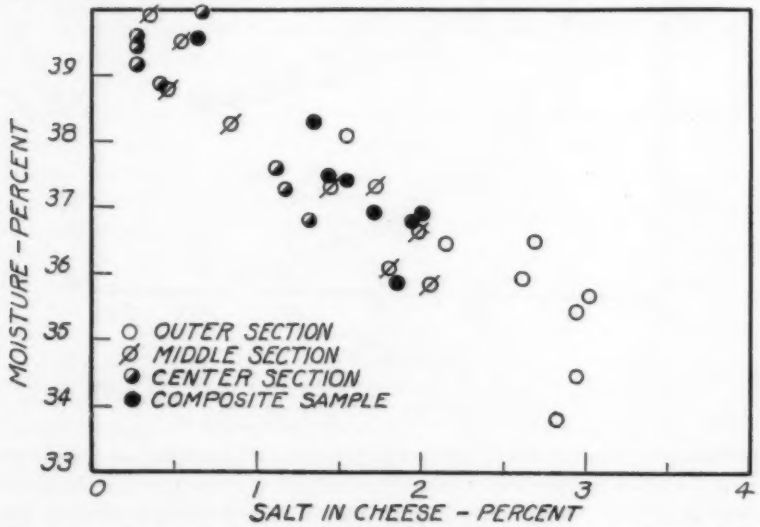

FIG. 2. THE RELATION BETWEEN THE MOISTURE AND SALT CONTENT OF BRICK CHEESE DURING THE FIRST WEEK OF CURING.

ceased after 18 hours when the temperature approximated 22° C. Differences in temperature and in acidity between the center and outer sections were greatest from four to six hours after dipping. At about the fourth hour after dipping, the rate of acid development of the center section decreased although the acidity of the outer section continued to increase fairly rapidly. At the tenth hour, both sections had the same acidity but after that time the acidity of the outer section was slightly greater than that of the center of the cheese.

The penetration of salt into the cheese is illustrated in Table 1. A 22%

TABLE 1

Salt penetration in a single lot of Brick cheese*

TIME AFTER DIPPING	SALT IN THREE SECTIONS OF CHEESE			SALT IN WHOLE CHEESE
	Outer	Middle	Center	
	Per cent	*Per cent*	*Per cent*	*Per cent*
32 hrs.	1.54	.34	.26	.63
44 hrs.	2.14	.43	.26	1.34
56 hrs.	2.62	.54	.26	1.54
68 hrs.	2.82	.83	.40	1.43
96 hrs.	2.94	1.45	.66	1.71
120 hrs.	2.94	1.71	.86	2.00
220 hrs.	2.65	2.05	1.31	1.85
1 mo.	2.68	2.62	2.34	2.59
3 mo.	2.54	2.59	2.62	2.56

* Salting began 20 hours after dipping. A 22 per cent sodium chloride brine was used.

sodium chloride brine was used and the cheese was placed in it 20 hours after dipping. At the time of salting the compositions of the various sections of the cheese were practically the same. The salt penetrated the cheese rapidly but not deeply. After 48 hours in the brine, the salt in the center section was only 0.4% although the salt in the outer section approximated its maximum value. The rate of diffusion of salt through the cheese seems to be a relatively slow process. The gradual increase in salt content of the whole cheese after the 68th hour, when the cheese was removed from the brine, may be accounted for by the use of weak brine solution for washing the cheese and by evaporation of moisture. The lot of cheese studied in Table 1 finally acquired more salt than was desirable. Slight irregularities in the data were caused by the necessity of analyzing different bricks of cheese in order to extend the period of observation.

An interesting fact concerning the relation between salt and moisture is illustrated in Figure 2. Each time the cheese or any section of it was analyzed for salt, the moisture content of the same sample was also measured. When the salt contents shown in Table 1 were plotted against moisture measurements it became evident that as the salt content increased the moisture in the cheese tended to decrease. This relationship agrees with

the observations of Jackson and Morris (4). The simple addition of salt would, of course, tend to decrease the percentage of moisture in the cheese. Calculations show, however, that the increase in salt could not alone account for the marked decrease in the moisture content of the cheese. Un-

TABLE 2

Influence of duration of salting in 22 per cent brine on the average acidity, moisture and salt content of three lots of Brick cheese

TIME OF ANALYSIS	ACIDITY			MOISTURE			SALT		
	Duration of salting			Duration of salting			Duration of salting		
	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.	24 hr.	48 hr.	72 hr.
	pH	*pH*	*pH*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Before salting	5.30	5.30	5.30	42.9	42.9	42.9	0	0	0
After salting	5.19	5.27	5.18	42.7	38.9	39.2	1.7	2.0	2.3
2 weeks	5.21	5.22	5.20	37.9	36.3	35.1	2.1	2.8	3.1
8 weeks	5.33	5.27	5.24	38.9	36.5	36.1	1.9	2.5	2.9

doubtedly the loss of moisture during the salting process must be attributed in part at least to the effect of the high osmotic pressure of the salt solution on the unsalted curd.

Influence of the duration of the salting treatment

The influence of the duration of the salting treatment was studied by dividing the cheese made from a single vat of mixed milk into three lots, one of which was salted for 24 hours, the second for 48 hours, and the third lot for 72 hours in 22% sodium chloride brine. This procedure was followed on three different days. The average of the measurements is shown in Table 2.

Acidity, as indicated by pH measurements, was not affected by the variations in the salting treatments to any marked extent. It is possible that the shortest period of salting might encourage an earlier breaking down of the protein. If this is true, then the slightly higher pH at 8 weeks of age in the cheese which was salted 24 hours might be regarded as evidence of this change.

The moisture content of all the lots decreased during the 8 weeks of observation. Those cheese in the lots salted for 48 and 72 hours lost more moisture than was desirable for the best ripening practices. Moisture content should be maintained at a level approximating 38% moisture to insure a moderately rapid ripening process. Slow ripening frequently results in the Cheddar type of flavor. This tendency was observed in the cheese salted for 72 hours.

The presence of salt may be expected to influence the ripening of the cheese, directly, through its influence on the biological agents themselves and, indirectly, through its influence on the physical properties of the curd,

such as moisture content and hardness of protein. Examination of the data of Table 2 indicates the natural tendency for the salt to increase as the duration of the salting period was lengthened. Increasing the salting period beyond 24 hours did not produce a proportionate increase in salt content. Apparently when the outer section of the cheese becomes highly salted then the increase in the salt content of the whole cheese depends upon the rate

TABLE 3

Effect of the duration of salting in 22 per cent brine on the average grade of the cheese*

CHARACTERISTIC	DURATION OF SALTING IN 22 PER CENT BRINE					
	24 hours		48 hours		72 hours	
	Grade	Criticism	Grade	Criticism	Grade	Criticism
Flavor	3.5	Slightly unclean	3.7	Slightly unclean	3.7	Salty Unclean
Body	3.2	Fairly smooth	2.8	Fairly long	3.4	Firm
Texture	3.7	Open Gassy	3.5	Open Gassy	3.3	Medium close

* Key to grades:—1—Excellent 3—Satisfactory 5—Very objectionable
2—Desirable 4—Objectionable 6—Unsalable

TABLE 4

Influence of method of salting on the average acidity, moisture and salt content of three lots of Brick cheese

TIME OF ANALYSIS	ACIDITY			MOISTURE			SALT		
	Dry salt	22 Per cent brine	26 Per cent brine	Dry salt	22 Per cent brine	26 Per cent brine	Dry salt	22 Per cent brine	26 Per cent brine
	pH	*pH*	*pH*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Before salting	5.33	5.33	5.33	41.5	41.5	41.5	0	0	0
After salting	5.16	5.17	5.22	39.8	39.1	38.8	1.6	2.1	2.2
2 weeks	5.19	5.22	5.19	37.0	35.7	35.4	2.2	2.7	2.7
8 weeks	5.36	5.30	5.30	36.1	36.0	32.3	1.4	2.4	2.7

of diffusion of the salt from the outside through the curd to the unsalted interior. This is illustrated by the fact that although the salt contents of the outer sections in these lots of cheese were 2.9, 3.5, and 3.9% immediately after the 24, 48, and 72 hour salting periods, still the percentages of salt at the center of the cheese at the same time were only 0.5, 0.5, and 0.7%, respectively.

Table 3, which shows the average grades given these lots of cheese, indicates that each salting treatment tends to excel in its beneficial influence in some characteristic. The shortest salting treatment produced the most desirable flavor; the 48 hour treatment developed the best body; and the 72 hour treatment induced the most desirable texture. The long salting periods produced cheese which were criticized for salty flavor, hard body,

and unnaturally white color. These defects would be less acceptable as a general rule to the consumer than the open texture for which the 24 hour treatment was criticized. The milk used in these trials was not of superior quality, which not only lowered the general level of grades but emphasized the danger of using light salting treatments with gassy milk.

Influence of the method of salting

The influence of the method of salting was studied by dividing the cheese from a single vat of mixed milk into three lots, one of which was salted by rubbing with dry salt once each day for two days; the second by placing in 22% brine for 48 hours; and the third by salting in 26% brine for 48 hours. This procedure was followed on three different days.

The 22% brine was selected for these trials because weaker concentrations usually developed bacterial growth and offensive odors after a few weeks at 60° F. (15.6° C.). The 26% brine was selected because to maintain its strength it was only necessary to keep undissolved salt in the bottom of the brine tank. Partially saturated solutions of salt in brine tanks vary widely from the concentrations desired, according to measurements which have been made in various factories.

Acidity measurements of the cheese are shown in Table 4 and are practically identical to those shown in Table 3. They reveal no significant differences which might be attributed to the method of salting.

Moisture measurements, shown in Table 4, indicate the severity of the treatment in the 26% brine. The moisture decreased constantly until the last observation at 8 weeks of age. Such a treatment, if it induced these changes under all conditions, would be highly undesirable. In its effect on moisture the dry-salt treatment approximated salting in 22% brine for 48 hours except that the moisture was held a little more tenaciously by the dry-salted cheese.

Salt measurements shown in Table 4 indicate two significant facts: first, the dry salting treatment does not incorporate salt as fast as the brine treatments used in these experiments; and second, the dry salting treatment induces greater irregularity in the rate of salt incorporation. As would be expected, the cheese salted in the 26% brine contained more salt than did either of the other lots.

The quality of the cheese made in these experiments is shown by the average grades in Table 5. There were practically no differences in the flavor of the three lots of cheese. Dry-salting and salting in 22% brine produced better body. The 26% brine caused the cheese to be white in color and too firm or curdy in body even after 8 weeks of curing. The grades on texture indicate a slight advantage in dry salting. This is surprising in view of the fact that the milk was of such inferior quality that gas holes were formed in all lots of cheese. This might be explained, how-

ever, by some observations which were made on the fermentation of lactose in the cheese. Differences in the browning of curd during drying for moisture tests attracted attention to the possibility of differences in the lactose content. Sanders (10) has related the browning of cheese at 100° C. to the lactose content and states that the intensity of color is proportional to the lactose present. By this browning test, cheese which was dry-salted showed little or no lactose present 68 hours after dipping while the brine salted

TABLE 5

Influence of method of salting on the average grade of Brick cheese*

CHARACTERISTIC	METHODS OF SALTING					
	Dry salting		48 hours in 22 per cent brine		48 hours in 26 per cent brine	
	Grade	Criticism	Grade	Criticism	Grade	Criticism
Flavor	3.2	Unclean	3.2	Unclean	3.3	Unclean
Body	2.8	Smooth	2.8	Slightly Curdy	3.0	Curdy
Texture	3.2	Open, Grassy	3.6	Open, Cracks	3.3	Open, Grassy

* Key to grades:—1—Excellent 3—Satisfactory 5—Very Objectionable
2—Desirable 4—Objectionable 6—Unsalable

TABLE 6

The effect of salting at 4 hours and at 20 hours after dipping on the average acidity, moisture, and salt content of three lots of Brick cheese

TIME OF ANALYSIS	SALTED 4 HOURS AFTER DIPPING			SALTED 20 HOURS AFTER DIPPING		
	Acid	Moisture	Salt in cheese	Acid	Moisture	Salt in cheese
	pH	*Per cent*	*Per cent*	*pH*	*Per cent*	*Per cent*
Before salting	5.88	45.0	0	5.30	42.9	0
After salting	5.48	42.6	2.52	5.27	38.9	2.03
2 weeks	5.34	38.5	3.37	5.22	36.3	2.77
8 weeks	5.38	37.3	2.94	5.27	36.5	2.50

TABLE 7

*Comparison of salting at 4 hours and at 20 hours after dipping on the penetration of salt into the cheese**

TIME BETWEEN DIPPING AND SALTING	SALT IN TWO SECTIONS OF CHEESE			SALT IN WHOLE CHEESE
	Outer	Center	Difference	
Analysis immediately after salting				
hours	*Per cent*	*Per cent*	*Per cent*	*Per cent*
4	4.09	.91	3.18	2.52
20	3.53	.53	3.00	2.03
Analysis two weeks after salting				
4	3.97	2.77	1.20	3.37
20	3.40	2.14	1.26	2.77

* Duration of salting was 48 hours for both lots.

cheese often showed browning in the outer section of the cheese immediately after salting, but, as the cheese aged, the color induced by drying gradually became less evident and finally disappeared. The highest salt concentrations were always associated with the most intense brown color. It is possible that further work might indicate that the rapid fermentation of the lactose in the dry salted lots inhibited somewhat the undesirable types of fermentation.

The effect of early salting

It is a common procedure in factory practice when the curd is of doubtful or inferior quality to salt the cheese on the day of making. Perhaps this custom originated from the knowledge that salt applied to the rind of Swiss cheese during the curing process tends to suppress gas formation.

In order to determine the effect of this treatment a single lot of curd was divided into two parts. One half of the curd was placed in the molds, drained 20 hours, then salted for 48 hours in 22% brine. The rest of the curd was drained for 4 hours only and then placed in the 22% brine for 48 hours. These comparisons were made on three different days. The effects of the treatment are summarized in Tables 6 and 7.

Every characteristic of the cheese was affected by early salting. The acidity development was definitely and permanently checked by the treatment. The contrast between the acidity of the experimental and the control lots indicates the danger of salting curd too soon if acid is required to control abnormal fermentations. It is known that acid develops rapidly in the cheese for about eight hours after dipping and much more slowly after that time. Early salting, therefore, should probably take place no sooner than 8 hours after dipping if the normal acid development is to be approximated. The moisture content of the early-salted cheese was higher than that of the control cheese at every measurement. This might be predicted from the trends of acid development which have been described. The salt content of the early-salted cheese was higher than that of the control. This may be attributed to the fact that the curd is not knit together in such an impervious mass 4 hours after dipping as it is 20 hours after dipping. Whey still drains freely from it at 4 hours. It is natural, therefore, to expect a more rapid exchange of salt and moisture especially during the early stages of salting. This assumption is verified by measurements of salt penetration.

The penetration of salt is indicated in Table 7. The data are based on analyses of the outer and center layers of the cheese. It is apparent that the cheese salted 4 hours after dipping absorbed salt more rapidly than did the control lot of cheese. The differences between the salt in the outer and center layers are practically the same for both lots of cheese at the two intervals of analysis.

The effect of early salting on the quality of the cheese at 8 weeks of age is also interesting. The flavor of the experimental and control lots were practically identical. The body of the early-salted cheese was inclined to be curdy and hard and in these respects distinctly inferior to the control cheese. The texture of the early-salted lots was slightly closer than that of the control lots. The early-salting treatment, under the conditions established in these trials, failed to inhibit gas development since a few gas holes were found in both the experimental and control lots. It is significant to note that the browning test for lactose showed nearly as much lactose after 8 weeks of curing as was present when the cheese was placed in the brine tank. As a whole, the curd salted 4 hours after dipping produced cheese which was inferior to that made from identical curd which was salted 20 hours after dipping.

DISCUSSION

The concentration of brine and the duration of the salting treatment both influence the loss of moisture from Brick cheese during curing. Excessive loss of moisture injures the body of the cheese and decreases the yield. It is difficult to reduce these losses by decreasing the salt in the brine much below 22% because weaker concentrations tend to undergo bacterial spoilage. It is highly essential, therefore, that the duration of the salting treatment be carefully regulated.

Cheese salted in 22% brine solution for 24 hours contained about two per cent salt. This treatment produced desirable body and texture. It is suggested that a saturated brine solution might be used for salting the cheese if the time of exposure did not exceed 24 hours. The use of saturated brine would eliminate the problem of regulating an unsaturated salt solution and would increase the capacity of the brine tank because of the reduction in the duration of salting.

There are several conditions under which this suggestion might actually cause trouble for the maker if it were followed. (1) The size of the loaves of cheese are an important factor in determing the salt penetration. Loaves larger than about five pounds should be salted a little longer while thin loaves should be removed from the brine sooner. (2) The recommendations are based on the assumption that the cheese is washed on alternate days with a two per cent salt solution. If clear water is used, salt is removed from the surface of the cheese. This might be a desirable treatment, for example, when the characteristic surface smear is slow in appearing, or when the cheese is over-salted in the brine tank. Occasionally factories crowd cheese in the brine tank so that the brine contacts only a small portion of each cheese. Obviously, any treatment recommended must be based on the assumption that the brine tank is large enough to provide space for floating the cheese. Crowding effects can be minimized to a certain extent

by frequent turning of the cheese, by judicious use of dry salt on exposed surfaces or by placing a weighted rack over the cheese in order to submerge it in the brine.

SUMMARY

Brick cheese curd was made by a commonly used method and subjected to various salting treatments. Measurements of acidity, moisture and salt content were made at regular intervals and the cheese were graded.

Salt penetrated the cheese quickly in the outer layer but eight weeks of curing elapsed before the salt content was practically uniform throughout the cheese. Long periods in the salt tank, highly concentrated brines, and early-salting increased the amount of salt in the cheese. Dry-salting was effective but not as uniform as brine-salting.

Desirable results were obtained when the Brick cheese absorbed salt to the extent of approximately 2% of its total weight. Excessive salting caused hard, curdy body, unnatural white color, slow ripening, loss of yield, and delayed lactose fermentation; low salt encouraged abnormal fermentations and caused weak body and open texture.

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THE GAS REQUIREMENTS OF MOLDS—I

A PRELIMINARY REPORT ON THE GAS REQUIREMENTS OF[1] *PENICILLIUM ROQUEFORTI* (VARIOUS STRAINS OF BLUE MOLD FROM CHEESE)

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This study was undertaken to ascertain the gas requirements of several strains of *Penicillium roqueforti* at different temperatures. In a previous study (13) of methods to increase the mold growth in blue veined cheese, it was realized that the present knowledge of the reaction of molds to gases was not sufficiently advanced for progress to be made by direct application of gases to cheese.

Existing data (3), (10), (33), (36) indicate that, in all probability, there are several varieties or strains of blue mold that are responsible for the ripening of blue veined cheeses, such as Roquefort, Gorgonzola and Stilton. Biourge (3), in addition to the name *P. roqueforti*, used *P. gorgonzola* and *P. stilton*, though it is doubtful that they can always be differentiated morphologically. As this study is primarily physiological, it must be appreciated that the Pencillia of closely similar morphology may differ widely in their metabolism (1), (8), (10), (11), (12), (13). It is for these reasons that a large and varied number of cultures, probably different strains, of *P. roqueforti* have been used in this investigation.

It is well known that *P. roqueforti* and many other molds produce CO_2 when grown in an enclosed space (10) (35). Durrell (6) showed that CO_2 in amounts up to 5 per cent stimulated mold growth (*Basisporium gallarum*). Plaz *et al.* (30) indicate an atmosphere of 15 per cent CO_2, produced by plant tissue or by a gas generator, as optimum for germination of spores of *Ustilago zeae*.

The inhibiting effect of CO_2 on the fungi has been widely studied (4), (5), (22), (27), (30), (32), (35). Various species of mold show wide differences in this respect (35). The work, as a whole, is difficult to correlate closely because of variations in the media, humidity, temperature of incubation, and the stage of germination of spores. Thom and Currie (35) have shown that *P. roqueforti* was able to grow at room temperature, with only slight restriction, in an atmosphere of 75 per cent CO_2 and 5 per cent O_2, while 21 other species of Penicillia suffered greater inhibition. Brown (4) found that *P. expansum* could grow in relatively high CO_2 pressures, an observation in agreement with the work of Thom and Currie (35). Early work by Chapin

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(5) showed that *P. glaucum*, an organism closely related to *P. roqueforti*, germinated in 90 per cent CO_2. These findings (4), (5), (35) are suggestive of the conclusion that, at room temperature, *P. roqueforti* and closely related strains are least inhibited by high concentrations of CO_2.

However, no molds grow in pure CO_2 (4) (5), but they are not killed (32) (34) and grow normally again when exposed to the air in suitable media and temperatures.

Brown (4) found that low temperatures increased the inhibiting power of CO_2 on the growth of mold and that the degree of inhibition was much more than could be accounted for by the greater solubility of CO_2 at the lower temperature. Other investigators (27), (32) also noted this property.

The media affect the growth of mold in concentrations of CO_2 which are partly inhibiting. Thus Brown (4) observed that a dilution of the nutrients retarded germination. Skovholts (32), investigating the growth of molds on bread, in inhibiting concentrations of CO_2, at low temperatures, compares his work with that of Moran *et al.* (27) and finds that under similar conditions a lower concentration of CO_2 will inhibit mold growth on meat. It would seem from other work (35) that the variety or strain of mold used could account for this difference. When the low pH at which molds will grow (14) (21) and the small change in pH that CO_2 can make in the medium (30) are noted, it is doubtful that pH could be a significant factor.

Another phase of study is that dealing with the effect of O_2 on mold growth in the absence of CO_2, that is air at low or high pressure, or with N_2 or some other inert gas as the diluent. Macy (24) working with 10 cultures of mold, 2 of which were Penicillia (*P. biforme* and *P. expansum*), shows that none of the molds grew on butter at room temperature when all the O_2 had been absorbed from the air by the alkaline pyrogallic acid reagent. The same molds, with one exception, growing under the same conditions but without the oxygen absorbent and with the pressure reduced by 25 inches, grew slowly. *P. expansum* showed the best growth and *P. biforme* grew as well as any of the other cultures. When the experiment was repeated over a 10 per cent aqueous solution of NaOH, better growth was obtained. Macy (24) states, "Apparently, a reduction in the amount of carbon dioxide does not seriously deter the growth of the species under observation."

Brown (4) shows that the amount of germination in a given time diminishes with increased concentration of O_2, but only very slowly, so that even in water quite a good germination results in 80 per cent O_2. Again, as the O_2 pressure is diminished, no appreciable effect is shown until very low concentrations of O_2 (0.1 per cent) are reached.

Karsner and Saphir (23) find that concentrations of O_2 of 76 per cent and more exercise a definite inhibitory effect on certain molds. In no case was the organism killed by the O_2 percentage employed, and on removal from the O_2 chamber, growth appeared to progress at a normal rate. The

production of pigments by the molds is not altered by growth in any of the O_2 concentrations used.

Thom and Currie (35) show by analysis that the percentage of the gases of Roquefort cheese are CO_2, 21.14 to 40.95; O_2, 2.42 to 7.00; and N_2, 54.52 to 73.44. The last paragraph of their summary is as follows: "A mixture of 75 per cent of carbon dioxide with air gives approximately 5 per cent of free oxygen. The close correspondence between the results of gas analysis and comparative culture, indicates that the low percentage of oxygen in the open spaces within the cheese accounts for the dominant activity of *Penicillium roqueforti* in Roquefort and related types of cheese."

It is impossible to determine from the existing literature whether or not it is the low percentage of O_2 or the high percentage of CO_2 which is the chief limiting factor in the growth of mold in Roquefort cheese.

The early fermentation in the manufacture and ripening of Roquefort and other cheeses is of the lactic type (7), (29). *Streptococcus lactis* (2), (20) and varieties of this organism (19), (20) as well as *S. citrovorus* and *S. paracitrovorus* (18), have all been shown to produce CO_2 in the fermentation of milk and other media. One of the striking and uniform findings of these authorities (2), (18), (19), (20) is the wide variation in CO_2 production of different cultures grown under the same conditions. Not only the different species but also separate cultures of the same organisms (19) (20) vary in this respect.

Ayers *et al.* (2) working with the souring of milk by the streptococci found that there appear to be two varieties of *S. lactis*, A. and B., the first of which produces CO_2 and NH_3 from peptone, while the second does not. Variety A., according to their results, appears to be more prevalent. In commercial starters both varieties have been found; some have variety B.

Van Slyke and Hart (37), who ripened two cheeses so that the CO_2 production could be measured, showed that the normal cheese produced .5 per cent of its weight as CO_2, while the chloroformed cheese produced only a trace of this gas. The experiment demonstrates that practically all the CO_2 produced in the cheese ripening process is due to the growth of microorganisms.

There are two recommendations as to the use of starters in Roquefort cheese making. Three recent publications (16), (17), (26) suggest the use of a good active starter to be used in relatively large quantities. The older French publication (25) does not mention the use of a starter but uses natural fermentation.

With the obvious possibility of controlling the production of CO_2 by fermentation in the making and ripening of Roquefort and other blue veined cheese, this study of the gas requirements of several strains of *P. roqueforti* was conducted.

CULTURES

The cultures of *P. roqueforti* used were mostly isolated from blue veined cheese, and are as follows:

ORIGIN OF CULTURES

Number of Cultures	*Origin*
1	*P. roqueforti* supplied by Dr. C. Thom about 1920.
15	Isolated from a Wensleydale Cheese made at the University of British Columbia.
16	" "
32	" "
33	" "
34	Isolated from a Wensleydale Cheese made by Rowntree, York, England.
37	" "
41	Isolated from a French Roquefort Cheese (Brand Civille).
43	Isolated from a N. K. Jensen mold powder supplied for inoculating Roquefort Cheese. (Imported from Denmark.)
3	Iowa Agr. Exp. Sta. Mold Culture No. 3 isolated from Danish Blue Cheese.
8	Iowa Agr. Exp. Sta. Mold Culture No. 8 isolated from French Roquefort Cheese.

Note: Cultures 3 and 8 were kindly supplied by Dr. B. W. Hammer, (38), (39).

Morphological and cultural studies of several of these cultures have been previously reported (8), (9), (10), (11), (12), (13), (14). As these cultures have been carried in stock for a number of years on malt agar slants, they were plated 3 times, a typical single colony being selected each time. Cultures 3 and 8, which were received from Dr. B. W. Hammer, were plated once. In the process of plating, contamination in the cultures was not observed. Incubation of the transfers was done at room temperature. Cultures of about one week of age were used to prepare water blanks with which to inoculate the plates.

MEDIA AND INCUBATION

Malt Agar: The malt agar used was the standard Difco product prepared as directed. The reaction was not changed.

Whey Agar: 5000 grams of sweet skim milk was renneted with 1 ml. of Hansen's rennet at about 90° F. (32.2° C.), and allowed to stand until well clotted. It was then cut with a knife and cooked by standing in water at about 120° F. (48.9° C.). The whey was drained off and agar added to the extent of 1½ per cent. The whole was heated in a steamer for 1 hour to dissolve the agar, then cooled to about 122° F. (50° C.) and the whites of 2 eggs added to 2000 ml. of media. The medium was then autoclaved and filtered.

Synthetic Media: For the 3 synthetic media the modified Czapek's salt solution as given by Naylor *et al.* (28) was used, plus the organic nutrients. The casein was put into solution with NaOH and adjusted to a reaction of pH 6.5 to 6.7 with HCl.

The media used were:

a. Salt solution with 3 per cent casein (impure). 1½ per cent agar.
b. Salt solution with 3 per cent casein (impure) and 1 per cent lactose. 1½ per cent agar.
c. Salt solution with 3 per cent peptone. 1½ per cent agar.

Preparation of Plates: To maintain uniformity between plates, 25 ml. of media were measured into 125 ml. Erlenmeyer flasks, plugged and sterilized at 15 lbs. for 30 minutes. The flasks were cooled somewhat and then the media poured on to sterile plates. By this method a thick uniform layer of medium was obtained. After the media solidified, wherever condensation of water on the lid of the petri dish was heavy, the lids were changed to prevent spreading the mold after inoculation.

Inoculation of Plates: A heavy loop of inoculum was transferred from the agar slant to a 100 ml. water blank and shaken well. One 2.5 mm. wire loop full of this aqueous dispersion of spores was used for inoculating the center of each plate.

Incubators: Special incubators which had sources of both heat and cold were used, so that a wide range of uniform temperatures was available.

Note: The range of temperature given in the tables is wide because the maximum and minimum readings for the experiment were recorded. Thus a 48° F. (8.9° C.) compartment opened only for a few seconds on a hot summer day will rise several degrees. Trials with self-recording thermographs show the temperature to be very uniform.

GASES USED

Carbon Dioxide: CO_2 was used from a commercial cylinder of the gas, which contained over 99.8 per cent of gas soluble in NaOH.*

Carbon Dioxide	99.80%
Carbon Monoxide	Trace
Oxygen	Trace
Nitrogen	00.15%
Moisture	00.03%

Nitrogen: N_2 was used from a commercial cylinder of the gas, which contained less than 0.2 per cent of CO_2 and 0.2 per cent O_2. The same cylinders were used for all experiments.

* Note: The Washington Liquid Gas Company give their certified analysis over a time of several years as follows:

Gas Chambers: 250 mm. Pyrex vacuum desiccators were used for growing the molds in the restricted air supply. By the use of wood blocks, it was possible to have each desiccator hold 22 plates each; *i.e.*, duplicates of the 11 cultures. It was found that the desiccators when properly greased maintained a very good vacuum. Controls were kept in tin trays.

Adding and Changing Gases: The desiccators, having been filled with the required inoculated plates, were evacuated to reduce the content of the air to the required fraction. The reduced pressure was measured with a manometer. The required gas was then added to the desiccator until atmospheric pressure was reached.

Example: Required, a mixture of 1 part of air to 3 parts of CO_2. Barometer pressure 700 mm. The desiccator was evacuated to a column of mercury of $\frac{700 \times 3}{4} = 525$ mm. CO_2 was then added to atmospheric pressure.

With the exception of the last experiment a daily change of gases was made. The method consisted in daily removing the lid of the desiccator, placing a tube to the bottom of the desiccator and sucking the air out for 3 minutes. The lid was placed on the desiccator which was then evacuated twice to 500 mm. each time, allowing air to enter at atmospheric pressure. Finally the chamber was evacuated to the calculated pressure and filled with the required gas.

It is not likely that the gas in the desiccator would remain constant for 24 hours, because of the growth of the mold, but the change in composition would be relatively slight, and the O_2 content would always tend to diminish rather than to increase.

Only in the last experiment were the gases adjusted as above but not changed.

MEASUREMENT OF COLONY

Whenever possible, the growth of the colony of mold was expressed in mm. which is the average diameter of 2 or more colonies, except those measurements marked * which were obtained from one colony. The difference between plates of the same culture of mold growth under the same conditions and for the same length of time usually did not exceed 2 to 3 mm. and in many cases they were identical. On poor media greater differences were sometimes observed.

Mathematical Expression of Growth Relationship: In all cases x has been used to represent the diameter of the control cultures in mm., y that of cultures grown in air diluted with CO_2 and z in air diluted with N_2. If the ratios $\frac{x}{y}$ or $\frac{x}{z}$ are greater than 1.0, growth in the experimental chamber is less than that in the control, and vice versa. Thus, when a culture grows to a diameter of 34 mm. in the air (control), and a diameter of 8.5 mm. in the

experimental culture with one part of air to 3 parts of CO_2 all other conditions being the same,

$$\frac{x}{y} = \frac{34}{8.5} = 4$$

Gas Analysis: Gas analysis was made with a William's gas analysis apparatus, which was accurate to 0.2 per cent.

Criticism of Methods: It is seldom that any biological measurement can be extremely accurate, and it therefore seems desirable to indicate one or 2 variables, which are beyond control in this study. A colony of mold growing on a medium in a petri dish never increases its diameter at a uniform rate. Also the greater the growth, the more pronounced will be the effect of the products of growth in depressing the rate of growth. Thus, in the last example where x = 34 mm. and y = 8.5 mm. in 5 days, in 7 days the restricted colony has a better chance to grow than the control, whose media is affected by more products of growth. Therefore, for the sake of comparison, an effort has been made to have the controls for each culture as uniform in size as possible. In several cases, measurements of young cultures are given for comparison.

TABLE I

Growth of strains of P. roqueforti in air and in air and CO_2 (1 to 3) at various temperatures

Approximate gas composition: 75% CO_2, 5.2% O_2, and 19.8% inert gas.
Media: Malt Agar.

Temperature of growth:	48° F. (8.9° C.)		70° F. (21.1° C.)			85° F. (29.6° C.)		
Temperature range:	42° F. to 52° F.		67° F. to 72° F.			83° F. to 86° F.		
Days growth:	14		5			4		
Culture:	x	y	x	y	$\frac{x}{y}$	x	y	$\frac{x}{y}$
1	36.5	No growth	42.5	20	2.13	27.5	6	4.58
15	26.5	" "	27.5	10	2.75	26	6	4.33
16	38	" "	43	17	2.53	25.5	6	4.25
32	37	" "	41.5	15.5	2.68	27	7	3.86
33	34	" "	39	16	2.44	24	6	4.00
34	38	" "	41.5	16.5	2.52	29	6	4.83
37	32.5	" "	34	8.5	4.00	17	Trace	
41	39	" "	45	22	2.05	31	Trace	
43	37.5	" "	30.5	18	1.69	26	6	4.33
3	39	" "	35*	20	1.75	22.5	Trace	
8	32.5	Trace	40*	16	2.50	30	7	4.29
Average	35.5		38.1	16.3	2.46	26		

x = Control diameter of colony in mm. of culture grown in air.
y = Diameter of colony in mm. of culture grown in 1 part of air to 3 parts of CO_2.
* Measurement from one colony.

Note: Mr. H. Fallscheer, Research Dairy Chemist, kindly made the gas analyses.

EXPERIMENTAL

The Effect of CO_2 on the Growth of P. roqueforti at Various Temperatures

In Tables I and II and Figs. 1, 2, and 3, the effects of 1 part of air to 3 parts of CO_2 (approximately 75 per cent CO_2, 5.2 per cent O_2 and 19.8 per cent inert gas) on the growth of *P. roqueforti* on malt agar at various temperatures are given. It has not been possible to give the relation of $\frac{x}{y}$ at 48° F. (8.9° C.), and with the small size of y at 85° F. (29.4° C.), it is doubtful whether or not the determination of $\frac{x}{y}$ has more than a general significance. The method at 85° F. (29.4° C.) is subject to some question particularly at the 7 day period, as there was considerable dehydration of the medium, which, because of the variable fit of petri dish lids, cannot be uniform.

TABLE II

Growth of strains of P. roqueforti in air and in air and CO_2 (1 to 3) at various temperatures

Approximate gas composition: 75% CO_2, 5.2% O_2, and 19.8% inert gas.
Media: Malt Agar.

Temperature of growth:	48° F. (8.9° C.)		70° F. (21.1° C.)			85° F. (29.6° C.)		
Temperature range:	42° F. to 52° F.		67° F. to 72° F.			83° F. to 86° F.		
Days growth	22		7			7		
Culture:	x	y	x	y	$\frac{x}{y}$	x	y	$\frac{x}{y}$
1	57	Slight growth	62.5	35	1.79	45.0	8.5	5.29
15	37	Just visible	44	20.5	2.15	42.5	9.5	4.47
16	56	Just visible	72	32.5	2.22	42.5	12	3.54
32	52	Just visible	57.5	31	1.86	39.5	14	2.82
33	49.5	Slight	57.5	34.5	1.67	35	10	3.50
34	52.5	Slight	66	32.5	2.03	41	8.5	4.82
37	42	Just visible	50.5	15.5	3.26	32.5	Trace	
41	60	No growth	67.5	42	1.61	47.5	Trace	
43	46.5	Just visible	45	35	1.29	42.5	8.5	5.00
3	51*	Just visible	54*	26.5	2.04	36.5	Trace	
8	47*	Just visible	62*	34	1.82	41.5	10.5	3.95
Average	50.1		58.1	30.8	1.97	40.6		4.18

x = Control diameter of colony in mm. of culture grown in air.
y = Diameter of colony in mm. of culture grown in 1 part of air to 3 parts CO_2.
All plates with the exception of Culture 41 showed definite growth of mold when held in air at 70° F. after the experiment.
* Measurement from one colony.

Tables I and II, for the 5 and 7 day period show:

(1) All controls grew normally.

(2) In all cases and at all temperatures CO_2 inhibited the growth of *P. roqueforti.*

(3) Temperature was a most important factor in inhibiting the growth of *P. roqueforti* in the presence of 1 part of air to 3 parts of CO_2.

(a) At 70° F. (21.1° C.) the average inhibiting effect of CO_2 on the growth of the cultures studied was such that the colonies were less than half the size of the controls at 5 days' growth and about half the size of the controls at 7 days' growth. Fig. 1. The average values for $\frac{x}{y}$ at 5 and 7 days' growth were 2.46 and 1.97, respectively. There was considerable variation between cultures.

(b) At 85° F. (29.4° C.) the inhibiting effect of CO_2 on the cultures studied was greater, little more than germination being recorded in many cases. Fig. 2. The average value for $\frac{x}{y}$ was between 4 and 5.

(c) At 48° F. (8.9° C.) the inhibiting effect of CO_2 was almost complete. Fig. 3.

(4) The variation between strains was considerable and will be discussed in a later paper.

The Effect of Higher Concentrations of CO_2 on the Growth of P. roqueforti at 70° F. (21.1° C.)

Table III gives the effect of 1 part of air to 6 parts of CO_2 on the growth of *P. roqueforti* on malt agar at 70° F. (21.1° C.). After the repetition of the work given in Table I and II, at 70° F. (21.1° C.) and 48° F. (8.9° C.), with substantially the same results, it was decided to increase the concentration of CO_2 at room temperature as an additional check. The results are given in Table III and show:

(1) Duplicate determinations run at different times checked quite closely.

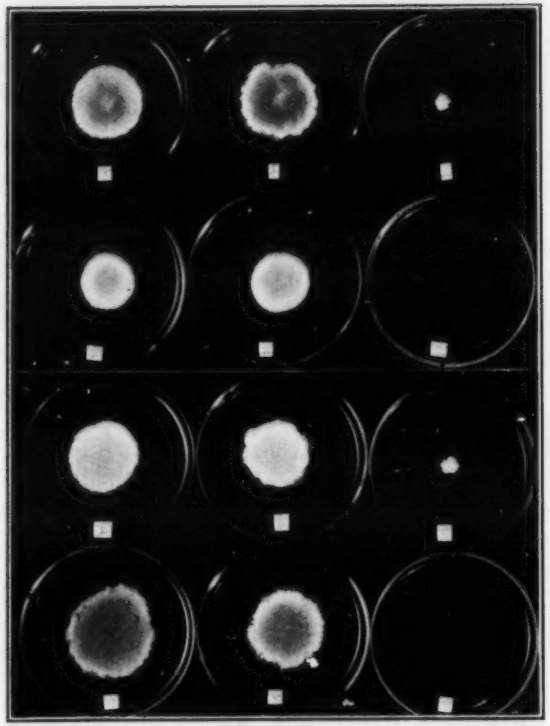
(2) Greater concentrations of CO_2 did not reduce the growth of the cultures in the same proportion as might be expected. The average values for $\frac{x}{y}$ at 7 days' growth were:

1 part of air to 3 parts of CO_2	1.97	Table II
1 part of air to 6 parts of CO_2	2.36	Table III

(3) The differences between strains were consistent.

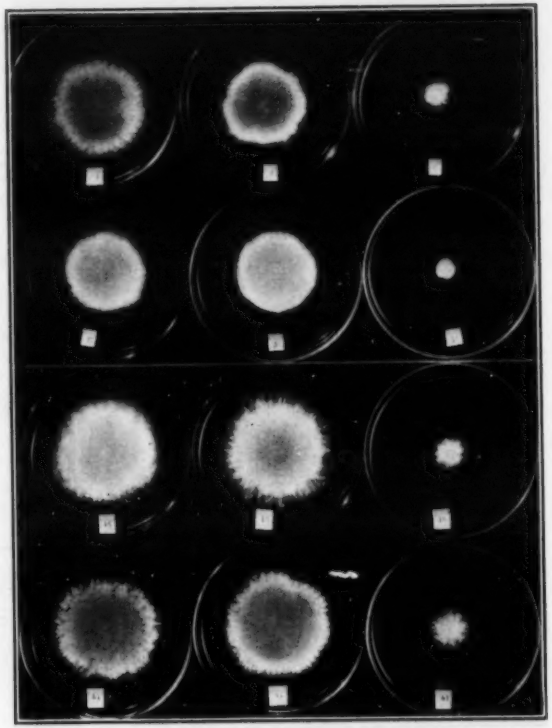
The Effect of Concentrations of CO_2 on the Growth of P. roqueforti after Germination at 48° F. (8.9° C.)

As no significant growth could be obtained at 48° F. (8.9° C.) in 1 part



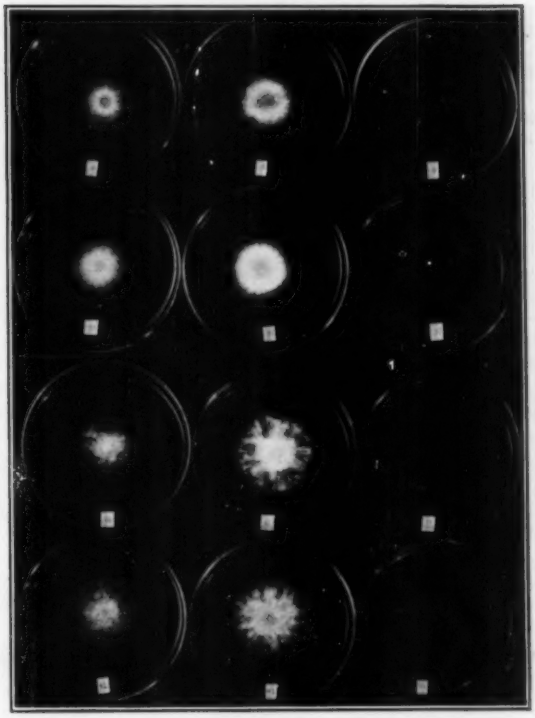
Control air | 1 part of air to 3 parts of N_2 | 1 part of air to 3 parts of CO_2

FIG. 2. The effect of N_2 and CO_2 on the growth of *P. roqueforti* at 85° F. (29.4° C.)



Control air | 1 part of air to 3 parts of N_2 | 1 part of air to 3 parts of CO_2

FIG. 1. The effect of N_2 and CO_2 on the growth of *P. roqueforti* at 70° F. (21.1° C.)



Control air | 1 part of air to 3 parts of N_2 | 1 part of air to 3 parts of CO_2

FIG. 3. The effect of N_2 and CO_2 on the growth of *P. roqueforti* at 48° F. (8.9° C.)

of air to 3 parts of CO_2, the inoculations on the plates were first germinated and grown to a small colony between 9 and 16 mm. in diameter, which required 48 hours at 70° F. (21.1° C.). Table IV. Column b. The experiment was then continued as in Tables I and II.

The results are given in Table IV and show:

(1) There was little or no increase in diameter of the colonies grown in 1 part of air to 3 parts of CO_2 in a period of 14 days. The controls grew normally.

(2) From this and the previous tables, it would appear that the inhibiting effect of CO_2 is about the same for germination and growth. In addition the appearance of the colonies grown in 1 part of air to 3 parts of CO_2 was quite different from that of the controls. The colonies were white in color and had a floccose growth more like *P. camemberti* than *P. roqueforti*. After a few hours in air

TABLE III

Growth of strains of P. roqueforti in air and in air and CO_2 (1 to 6) at 70° F.

Approximate gas composition: 84.8% CO_2, 3.0% O_2, and 12.2% inert gas.						
Media: Malt Agar.						
Temperature of growth:			70° F. (21.1° C.)			
Temperature range:			66° F. to 72° F.			
Days growth:		7			7	
Cultures:	x	y	$\frac{x}{y}$	x	y	$\frac{x}{y}$
1	56	26.5	2.11	57	26.5	2.15
15	45.5	16	2.84	44.5	15.5	2.87
16	61.5	25.5	2.41	66	26.5	2.49
32	58	24.5	2.36	56.5	21.5	2.63
33	58.5	27	2.17	50	25.5	2.35
34	66	22.5	2.93	65	22.5	2.89
37	49	15	3.27	48.5	14.5	3.35
41	56	29.5	1.90	54	29.5	1.83
43	44.5	27.5	1.62	45*	25.5	1.76
3	52	23.5	2.21	52.5	21.5	2.44
8	59.5	28	2.13			
Average	55.1	24.1	2.36	54.9	22.9	2.48

x = Control diameter of colony in mm. of cultures grown in air.
y = Diameter of colony in mm. of cultures grown in 1 part of air to 6 parts of CO_2.
* Measurement from one colony.

at room temperature, the colonies took on their characteristic blue green color.

The Effect of CO_2 on the Growth of P. roqueforti, Different Media Being Used

To establish the general inhibiting effect of CO_2, different media must be used. A set of 10 strains of *P. roqueforti* was grown on whey agar, and also 4 selected cultures on 3 synthetic media. It is to be expected from previous work (10) (11) (12) that the culture strains used would vary with media, but it was hoped that the general trend could be determined.

Table V, with the exception of the medium, is comparable to Table III and shows:

(1) The general trend of growth inhibition by 1 part of air to 6 parts of CO_2 was of the same magnitude.

(2) Certain cultures, for example 3 and 15, deviated widely in their $\frac{x}{y}$ values from the results with malt agar. The difference was in part due to their finding whey agar a better medium for growth, as is shown by the controls.

Table VI and Figs. 4 and 5 show the effect of 1 part of air to 3 parts of CO_2 at 48° F. (8.9° C.) and 70° F. (21.1° C.) on 3 synthetic media.

TABLE IV

Growth of strains of P. roqueforti in air and in air and CO_2 (1 to 3) at 48° F. after a germination period of 2 days at 70° F.

Approximate gas composition: 75% CO_2, 5.2% O_2, and 19.8% inert gas.
Media: Malt Agar.
Temperature of growth: 48° F. (8.9° C.)
Temperature range: 44° F. to 50° F.
Days growth after germination: 14

	Control			1 part of air to 3 parts of CO_2		
Culture:	a	b	a – b	a	b	a – b
1	69	13	56	15	13	2
15	53	12	41	13	11	2
16	81.5	16	65.5	17.5	16	1.5
32	76.5	14	62.5	14.5	13	1.5
33	69	14	55	15.5	14.5	1
34	72	12	60	12	10.5	1.5
37	57.5	10.5	47	12	10.5	1.5
41	79.5	14	65.5	13	12	1
43	66.5	14.5	52	13.5	13.5	–
3	58.5	9	49.5	13	10	3
8	75	13.5	61.5	16.5	13.5	3
Average	68.9	13.0	56.0	14.1	12.5	1.6

a = Diameter of colony at the end of 14 days growth.
b = Diameter of colony after germination period.

Remarks: The mold growth in 1 part of air to 3 parts of CO_2 had the appearance of *P. camemberti*, being both white in color and floccose. After a few hours in air at room temperature the colonies took on their characteristic blue green color.

TABLE V

Growth of strains of P. roqueforti in air and in air and CO_2 (1 to 6) at 70° F.

Approximate gas composition: 84.8% CO_2, 3.0% O_2, and 12.2% inert gas.
Media: Whey Agar.
Temperature of growth: 70° F. (21.1° C.)
Temperature range: 67° F. to 72° F.
Days growth: 7

Culture:	x	y	$\frac{x}{y}$
1	59	27	2.19
15	52.5	13.5	3.89
16	66	23.5	2.81
32	61	19.5	3.13
33	59.5	26.5	2.25
34	69	20	3.45
37	67.5	18	3.75
41	56.5	27.5	2.06
43	41.5	24	1.73
3	41	24	1.71
Average	57.4	22.4	2.69

x = Control diameter of colony in mm. of culture grown in air.
y = Diameter of colony in mm. of cultures grown in 1 part of air to 6 parts of CO_2.

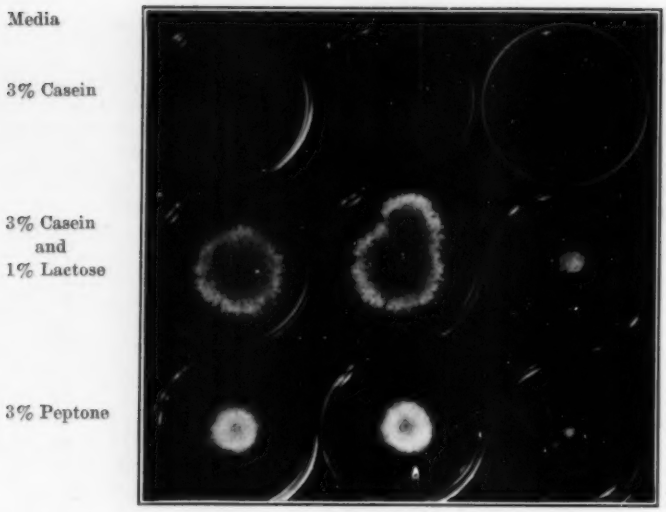


FIG. 4. The effect of N_2 and CO_2 on the growth of *P. roqueforti* Culture 8 at 70° F. (21.1° C.) on three synthetic media.

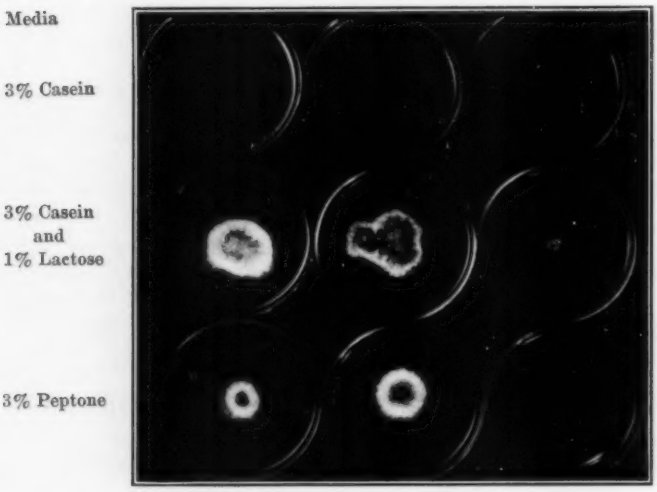


FIG. 5. The effect of N_2 and CO_2 on the growth of *P. roqueforti* Culture 16 at 70° F. (21.1° C.) on three synthetic media.

TABLE VI

Growth of strains of P. roqueforti in air and in air and CO_2 (1 to 3) at various temperatures and in various media

Approximate gas composition: 75% CO_2, 5.2% O_2, 19.8 inert gas.						
Temperature of growth:	48° F. (8.9° C.)			70° F. (21.1° C.)		
Temperature range:	45° F. to 57° F.			69° F. to 72° F.		
Days growth:	17			7		
	3% Casein					
Cultures:	x	y	$\frac{x}{y}$	x	y	$\frac{x}{y}$
16	50.5	No growth		58	25.5	2.28
37	44	" "		57	26.5	2.15
41	29*	" "		50*	18	2.78
8	43.5	Trace		51	22.5	2.27
Average	41.8			54.0	23.7	2.37
	3% Casein and 1% Lactose					
Cultures:	x	y	$\frac{x}{y}$	x	y	$\frac{x}{y}$
16	56	Trace		67	25	2.68
37	50.5	"		61.5	27	2.28
41	47.5	No growth		53	24	2.21
8	46.5	Trace		55.5	28.5	1.95
Average	50.1			59.3	26.1	2.28
	3% Peptone					
Cultures:	x	y	$\frac{x}{y}$	x	y	$\frac{x}{y}$
16	29*	No growth		35.5	24	1.48
37	21.5	" "		28.5	No growth	
41	No growth	" "		35.0*	" "	
8	" "	" "		26.5	" "	
Average				31.4		

* Measurement from one colony.

x = Control average diameter of colony in mm. of cultures grown in air.

y = Average diameter of colony in mm. of cultures grown in 1 part of air to 3 parts of N_2.

(1) The control cultures grew poorly on 3 per cent peptone, fairly well in 3 per cent casein, and well in 3 per cent casein plus 1 per cent lactose.

(2) As in all previous tables, 48° F. (8.9° C), 1 part of air to 3 parts CO_2 almost completely inhibited growth.

(3) At 70° F. (21.1° C.) the inhibiting effects of 1 part of air and 3 parts of CO_2 were of the same order of magnitude as previous results. In 3 cases there was no growth in 3 per cent. peptone which at best is a very poor medium. Figs. 4 and 5.

The Effect of N_2 on the Growth of P. roqueforti at Various Temperatures

Tables VII and VIII, Figs. 1, 2, and 3 give the results of the effect of

TABLE VII

Growth of strains of P. roqueforti in air and in air and N_2 (1 to 3) at various temperatures

Approximate gas composition: 5.2% O_2 and 94.8% inert gas.									
Media: Malt Agar									
Temperature of growth:	48° F. (8.9° C.)			70° F. (21.1° C.)			85° F. (29.6° C.)		
Temperature range:	44° F. to 52° F.			68° F. to 76° F.			83° F. to 86° F.		
Days growth:	10			5			4		
Culture:	x	z	$\frac{x}{z}$	x	z	$\frac{x}{z}$	x	z	$\frac{x}{z}$
1	20.5	27	.76	36	38*	.95	27.5	16	1.72
15	13.0	20	.65	28.5	30	.95	26	18.5	1.41
16	19.0	31	.61	39	40.5	.96	25.5	19.5	1.31
32	18.5	29	.64	36	37	.97	27	23	1.17
33	18	24.5	.74	34.5	35.5	.97	24	18.5	1.30
34	19.5	27.5	.71	37.5	42.5	.88	29	21	1.38
37	18.5	22.5	.82	30.5	30.5	1.00	17	12	1.42
41	19	27.5	.69	39	41	.95	31	23	1.35
43	24.5	30	.82	30	29	1.03	26	19	1.37
3	20.5	25	.82	36	32	1.13	22.5	16.5	1.36
8	16	21.5	.74	36	35	1.03	30	25	1.20
Average	18.8	26	.73	34.8	35.6	.98	26	19.3	1.36

x = Control diameter of colony in mm. of cultures grown in air.

z = Diameter of colony in mm. of cultures grown in 1 part of air to 3 parts of N_2.

* Measurement from one colony.

1 part of air to 3 parts of N_2 (approximately 5.2 per cent O_2 and 94.8 per cent inert gas) on the growth of *P. roqueforti* on malt agar at various temperatures. As with CO_2, the method at 85° F. (29.4° C.) is subject to some question, particularly at the 7-day period, as there was considerable dehydration of the medium since the fit of petri dish lids was not uniform.

Table VII and VIII and Figs. 1, 2, and 3 show:

(1) All controls grew normally.

(2) The depression or acceleration of growth, in an atmosphere of 1 part of air to 3 parts of N_2, appears to be a function of temperature.

(a) At 70° F. (21.1° C.) there was no significant difference between the size of the colonies grown in air and those grown in 1 part of air and 3 parts of N_2. The average values for $\frac{x}{z}$ at 5 days was .98, and for 7 days 1.05. Fig. 1.

(b) At 85° F. (29.4° C.) and 4 days' growth, the control colonies were significantly larger than those grown in 1 part of air and 3 parts of N_2. Fig. 2. The medium was somewhat dehydrated at 7 days.

TABLE VIII

Growth of strains of P. roqueforti in air and in air and N_2 (1 to 3) at various temperatures.
Approximate gas composition: 5.2% O_2 and 94.8% inert gas.
Media: Malt Agar.

Temperature of growth:	48° F. (8.9° C.)			70° F. (21.1° C.)			85° F. (29.6° C.)		
Temperature range:	42° F. to 52° F.			68° F. to 76° F.			83° F. to 86° F.		
Days growth:	14			7			7		
Culture:	x	z	$\frac{x}{z}$	x	z	$\frac{x}{z}$	x	z	$\frac{x}{z}$
1	36.5	42.5	.86	55	58*	.95	45	33.5	1.34
15	26.5	30.5	.87	44	43	1.02	42.5	37	1.15
16	38	51	.75	65.5	65.5	1.00	42.5	41.5	1.02
32	37	48.5	.76	61.5	54.5	1.13	39.5	43	.92
33	34	41	.83	55	54	1.02	35	36.5	.96
34	38	48	.79	62.5	67	.93	41	48	.85
37	32.5	34.5	.94	48.5	44.5	1.09	32.5	33.5	.97
41	39	45.5	.86	64	59	1.09	47.5	43.5	1.09
43	37.5	42	.89	45.5	41.5	1.10	42.5	32	1.33
3	39	39	1.00	55.5	48	1.16	36.5	36	1.01
8	32.5	37.5	.87	58	53	1.09	41.5	46.5	.89
Average	35.5	41.8	.86	55.9	53.5	1.05	40.6	39.2	1.05

x = Control diameter of colony in mm. of cultures grown in air.
z = Diameter of colony in mm. of cultures grown in 1 part of air to 3 parts of N_2.
* Measurement from one colony.

(c) At 48° F. (8.9° C.), both after 10 and 14 days' growth, respectively, the control colonies were smaller, showing a definite accelerating effect of 1 part of air and 3 parts of N_2. The average values for $\frac{x}{z}$ at 10 and 14 days' growth were .73 and .86, respectively. Thus the accelerating effect was more marked in the younger colonies. There was a considerable difference among some of the strains. Fig. 3.

(3) Variation among strains will be discussed in a later paper.

The Effect of High Concentrations of N_2 on the Growth of P. roqueforti at 48° F. (8.9° C.) and 70° F. (21.1° C.)

Since the previous experiment, Tables VII and VIII, showed no inhibition, but rather an acceleration of the growth of *P. roqueforti* at 48° F. (8.9° C.) and normal growth at 70° F. (21.1° C.), it was considered desirable to repeat the experiment with a few cultures at a higher concentration of N_2. One part of air and 9 parts of N_2 (approximately 2.1 per cent O_2 and 97.9 per cent inert gas) were selected as the atmosphere and the results are given in Table IX, which shows:

(1) At 70° F. (21.1° C.), both at 4 and 7 days the control grew better than the cultures in 1 part air and 9 parts N_2. This inhibition

TABLE IX

Growth of strains of P. roqueforti in air and in air and N_2 (1 to 9) at various temperatures.

Approximate gas composition: 2.1% O_2 and 97.9% inert gas.
Media: Malt Agar.

Temperature of growth:	48° F. (8.9° C.)			80° F. (21.1° C.)		
Temperature range:	45° F. to 56° F.			69° F. to 72° F.		
Days growth:	13			4		
Culture	x	z	$\frac{x}{z}$	x	z	$\frac{x}{z}$
16	14.33	19	.75	29	23.5	1.23
37	13.33	15	.89	24.5	18.5	1.32
41	17.33	24	.72	33	26.5	1.25
8	17.33	17.5	.99	30.5	23.5	1.30
Average	15.6	18.9	.84	29.3	23.5	1.28
Days growth:		17			7	
Culture	x	z	$\frac{x}{z}$	x	z	$\frac{x}{z}$
16	32.33	37	.87	64.5	52.5	1.23
37	27	25	1.08	48	35	1.37
41	35.66	39	.91	67	53.5	1.25
8	33.33	30.5	1.10	64	43	1.49
Average	32.1	32.9	.99	60.9	46	1.34

x = Control average diameter of colony in mm. of cultures grown in air.
z = Average diameter of colony in mm. of cultures grown in 1 part of air and 9 parts of N_2.

was relatively slight. The average values for $\frac{x}{z}$ for the 4 cultures at 4 and 7 days were 1.28 and 1.34 respectively.

(2) At 48° F. (8.9° C.) at 13 days the controls had not grown as well as the experimental cultures. At 17 days, growth was about the same, 2 cultures having an $\frac{x}{z}$ value under 1.00, while the others were over 1.00. The average value for $\frac{x}{z}$ for the 4 cultures at 13 and 17 days were .84 and .99 respectively. Fig. 6, taken from a similar series at 18 days' growth for culture 16 at 48° F. (8.9° C.), gives a remarkable example of how well this culture grew in an atmosphere of what must be 2 per cent of O_2 or less, and the remainder largely N_2.

The Effect of N_2 on the Growth of P. roqueforti, Different Synthetic Media Being Used

To establish the general effect of N_2, different media were used. As previously stated, it was to be expected that various media might affect the

different cultures in different ways (10) (11) (12). However, it was hoped to determine whether or not the general trend was the same.

Table X, Figs. 4 and 5, shows the effect of 1 part of air and 3 parts of

TABLE X

Growth of strains of P. roqueforti in air and in air and N_2 (1 to 3) at various temperatures and in various media

Approximate gas composition: 5.2% O_2 and 94.8% inert gas.						
Temperature of growth:	48° F. (8.9° C.)			70° F. (21.1° C.)		
Temperature range:	45° F. to 57° F.			68° F. to 72° F.		
Days growth:	17			7		
	3% Casein					
Culture:	x	z	$\frac{x}{z}$	x	z	$\frac{x}{z}$
16	50.5	56	.90	58	60.5	.96
37	44	48	.92	57	58	.98
41	29*	No growth		50*	38*	1.32
8	43.5	54*	.81	51	47	1.09
Average	41.8		.88	54	50.9	1.09
	3% Casein and 1% Lactose					
Culture:	x	z	$\frac{x}{z}$	x	z	$\frac{x}{z}$
16	56	63	.89	67	68	.99
37	50.5	50	1.01	61.5	60	1.03
41	47.5	60*	.79	53	51	1.04
8	46.5	51	.91	55.5	49	1.13
Average	50.1	56	.90	59.3	57	1.05
	3% Peptone					
Culture:	x	z	$\frac{x}{z}$	x	z	$\frac{x}{z}$
16	29*	35.5	.82	35.5	37	.96
37	21.5	23.5	.92	28.5	36	.79
41	No growth	33*		35*	42*	.83
8	No growth	27*		26.5	32.5	.82
Average				31.4	36.9	.85

x = Control average diameter of colony in mm. of cultures grown in air.
z = Average diameter of colony in mm. of cultures grown in 1 part of air to 3 parts of N_2.
* Measurement from one colony.

N_2 at 48° F. (8.9° C.) and 70° F. (21.1° C.) on the 3 synthetic media.

(1) The controls were the same as in Table VI, and as previously stated, the cultures grew poorly on 3 per cent peptone, fairly well on 3 per cent casein, and well on 3 per cent casein plus 1 per cent lactose.

(2) At 70° F. (21.1° C.) for 3 per cent casein and 3 per cent casein plus 1 per cent lactose, there was no significance in the size of

the colonies growing in air and those growing in 1 part of air and 3 parts of N_2. The cultures when grown in 3 per cent peptone showed a distinctly better growth in 1 part of air and 3 parts of N_2. This effect was noticed only with 3 cultures. The $\frac{x}{z}$ values for the 4 cultures in malt agar, Table VIII, and the casein and casein plus lactose were of very similar values, with the exception of culture 41 on casein. With the smaller growth on peptone the results were not comparable.

(3) At 48° F. (8.9° C.) for 3 per cent casein and 3 per cent casein plus 1 per cent lactose, accelerated growth was found in 1 part of air to 3 parts of N_2. The values for $\frac{x}{z}$ are of the order of magnitude previously found with malt agar (Table VIII) when allowance is made for the longer growth period. The cultures grew so badly in peptone that little significance can be attached to the results.

(4) The use of different media, either at 48° F. (8.9° C.) or 70° F. (21.1° C.), did not alter the general trend as found in Tables VII and VIII.

The Capacity of P. roqueforti to Grow in Air Low in Oxygen at 48° F. (8.9° C.) and 70° F. (21.1° C.)

The strains of *P. roqueforti* were grown as in previous experiments, with the difference that gas content was not adjusted daily. In 4 cases the CO_2 produced by growth of the mold was absorbed by placing a porridge dish of concentrated NaOH solution in the bottom part of the desiccator; in the other 2 cases no CO_2 absorbent was used. The same 11 cultures in dulicate, 22 plates in all, were used in each desiccator. Though triplicate colonies of the control checked well, a few of the duplicate colonies in the restricted air supply did not check. This condition could possibly be accounted for by the petri lids not being uniform, which would affect the air above the colony.

Table XI shows:

(1) There was no CO_2 in the desiccator at the end of the period where the NaOH solution was used as absorbent. CO_2 was present in considerable quantity where no absorbent was used.

(2) There was no significant quantity of O_2 left in the desiccators, with the exception of the desiccator in which the cultures were grown at 48° F. (8.9° C.) in the absence of the CO_2 absorbent. However, the 2 other desiccators in which the cultures were grown at 48° F. (8.9° C.) showed a slightly higher O_2 content (.3 per cent) than those at 70° F. (21.1° C).

TABLE XI

The average growth of eleven strains of P. roqueforti in air and in air and nitrogen on malt agar

Initial Atmosphere	Absorbent	Temperature of Incubation	Day's Growth	x	z	$\frac{x}{z}$	Gas Analysis CO_2 %	Gas Analysis O_2 %	Inert Gas
1 part of air to 3 parts N_2	NaOH	70° F. (21.1° C.)	8	62.3	49.8	1.26	Nil	.1	99.9
" "	NaOH	48° F. (8.9° C.)	20	67.9	49.2	1.39	Nil	.3	99.7
1 part of air to 9 parts N_2	NaOH	70° F. (21.1° C.)	8	62.3	37.2	1.69	Nil	Nil	100
" "	NaOH	48° F. (8.9° C.)	20	67.9	47.1	1.45	Nil	.3	99.7
" "	Nil	70° F. (21.1° C.)	8	62.3	36.4	1.73	2.5	Trace	97.5
" "	Nil	48° F. (8.9° C.)	20	67.9	42.1	1.62	3.0	1.4	95.6

x = Control average diameter of colonies in mm. of cultures grown in air.

z = Average diameter of colonies in mm. of cultures grown in an atmosphere diluted with N_2.

Range of temperature for 48° F. (8.9° C.) incubator 46° F. to 68° F.

Note: The 68° F. is due to refrigeration being off for 30 hours.

Range of temperature for 70° F. (21.1° C.) incubator 67° F. to 72° F.

(3) The average growths must be discussed in pairs; *i.e.*, lots with the same initial atmosphere but varying in temperature of growth.

(a) In an initial atmosphere of 1 part of air to 3 parts of N_2, the average value for z at both temperatures was practically the same. Thus the mold colonies at 48° F. (8.9° C.) and 70° F. (21.1° C.) stopped growth at the same size in the restricted air supply, while the controls grew on, reaching the greater size at 48° F. (8.9° C.).

(b) With an initial atmosphere of 1 part of air to 9 parts of N_2 held over NaOH, the average z values showed better growth at the lower temperature. It is probable that the colonies at the lower temperature got more O_2 by absorption and that the type of growth was more spreading.

(c) With an initial atmosphere of 1 part of air to 9 parts of N_2, with no CO_2 absorption, the average z values showed slightly better growth at the lower temperature. However, had all the O_2 been used up at the lower temperature, the z values would probably have been similar to the last pair.

(4) Discussing the second and third pair at the same initial atmosphere, with and without a CO_2 absorbent, it was found the removal

of CO_2 increased growth, which increase was marked at the lower temperature.

DISCUSSION

It must be pointed out that in no cases do these data conflict with the previous data cited, but they do conflict with the statements explaining such data (24) (35). Had Thom and Currie (35) used N_2 as well as CO_2 to dilute the O_2 of the air in their Novy jars they would have found very different results with the former gas than they did with the latter. Also, had they used the ripening temperature of Roquefort cheese (48° F.) (8.9° C.) for incubating their cultures, they would have been more impressed with the inhibiting effect of CO_2. The data herein reported show clearly that the gas requirements of *P. roqueforti* are functions of both concentration and temperature and vary with different gases. The inhibiting effect of CO_2 varies greatly with temperature. The O_2 requirements appear, also, to be a function of temperature. Whether or not the variation between strains is associated with maximum, minimum and optimum temperatures of their growth in air, is yet to be determined and will be taken up more fully in a later paper. Furthermore, the work points to maximum, minimum and optimum temperatures of growth as being variable with gas supply. In this respect it must be pointed out that the findings of Thom and Currie (35) regarding the restriction of growth by CO_2 on a large number of Penicillia might produce quite different results at another temperature. Let it be assumed for the moment that the findings in this study apply to all Penicillia. Then the organisms used by Thom and Currie (35), which were grown in high concentrations of CO_2 at room temperature, would either be at, above or below their optimum temperature of growth in air. Thus an organism with a high optimum temperature of growth would naturally be more restricted by CO_2 at room temperature than one which has its optimum at the room temperature used.

From the standpoint of blue veined cheese making and ripening the results are of fundamental importance. The presence of CO_2 rather than the absence of O_2 is the limiting factor in the growth of *P. roqueforti* in the cheese, particularly as the ripening temperature of these cheeses is between 40° F. (4.4° C.) and 50° F. (10° C.). A cheese is punched, pricked or skewered, not to admit O_2 but rather to allow CO_2 to escape. The production of CO_2 by lactic bacteria is undoubtedly carried on in the cheese (37). The extent of CO_2 production will largely depend on the type of organisms present (2) (18) (19) (20). In the selection of a starter for cheese making it is possible to choose one of the type A starters (2) which will produce large quantities of CO_2, having an inhibiting effect on the growth of *P. roqueforti*. On the other hand, selection of a starter of the type B starter (2) will result in the production of much less CO_2, a condition favorable

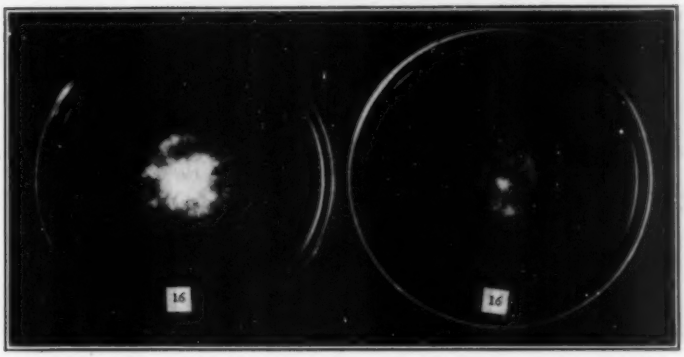


FIG. 6

1 part of air to 9 parts of N_2 — Control air

The effect of N_2 on the growth of *P. roqueforti* Culture 16 at 48° F. (8.9° C.)

for the growth of *P. roqueforti*. The latter starter also would allow for the use of the more desirable lower cheese ripening temperatures. With more attention to the selection of starters by their CO_2 production, continuous success with blue veined cheeses, Roquefort, Gorgonzola, Stilton, and Wensleydale, will probably be attained.

It is to be regretted that in the experiments presented in this paper as well as in others cited, (4) (15) (24) (32) too close attention to the practical application results in limitation of fundamentals. If this work could be continued from the extreme maximum temperature of growth in air to the extreme minimum temperature of growth in air, with variations in N_2, O_2 and CO_2 content, curves could be drawn for $\frac{x}{y}$ and $\frac{x}{z}$ from zero to 100 per cent concentrations.

CONCLUSION

(1) CO_2 inhibits the growth of several strains of *P. roqueforti* in several different media. The degree of inhibition is uniform with the medium but varies with concentration of CO_2 and temperature. With 75 per cent CO_2 the effects are:
   (a) At 85° F. (29.4° C.) the inhibition is very considerable, such that little more than germination takes place.
   (b) At 70° F. (21.1° C.) the inhibition is only partial, the colonies being about half the size of those grown in air.
   (c) At 48° F. (8.9° C.) the inhibition is almost total.

(2) The reduction of the O_2 by added N_2 affects the growth of several strains of *P. roqueforti* in several different media.

(a) At 85° F. (29.4° C.) in an atmosphere of 1 part of air to 3 parts of N_2 the inhibition is slight.

(b) At 70° F. (21.1° C.) the cultures grow in 1 part of air to 3 parts of N_2 as well as in air.

(c) At 48° F. (8.9° C.) the growth in 1 part of air to 3 parts of N_2 is considerably accelerated over that in air. Even in 1 part of air to 9 parts of N_2 there is no inhibition.

(3) Where CO_2 is removed by NaOH the strains of *P. roqueforti* growing in low O_2 pressure, removed all but a trace of O_2, at both 70° F. (21.1° C.) and 48° F. (8.9° C.).

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THE EFFECT OF FAT CONTENT ON OXIDIZED FLAVOR IN MILK AND CREAM

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Practical experience and observation has shown that milk plants which produce two grades of pasteurized milk generally encounter much more difficulty with oxidized flavor in the high-fat premium-quality grade than in the standard grade. Roland, Sorensen, and Whitaker (1) studied oxidized flavor in commercial pasteurized milk and concluded that both the bacteriological quality of the milk and its fat content were related to the flavor defect. Tracy and Ruehe (2) reported the results of adding a small quantity of a lactic acid solution of copper oxide to four milk products ranging in fat content from skim milk to 4.9%. In the series the oxidized flavor became more apparent as the fat content increased. They found that skim milk did not develop oxidized flavor but developed metallic flavor.

In order to study systematically the effect of fat content on the sensitivity of milk and cream to copper-induced oxidized flavor, a series of laboratory experiments were undertaken. Samples of milk and its products of various fat contents obtained by separation and recombination were tested for sensitivity by pasteurizing them in presence of definite areas of metallic copper surfaces.

PROCEDURE

A commercial grade of 17 gauge copper wire was used as a source of metallic copper. Pieces varying in length from 7.5 inches to 60 inches were cleaned by abrasion with powdered pumice stone on a piece of moistened blotting paper and then rinsed in cold water. Inasmuch as freshly cleaned copper was found to discolor in a relatively short time in the laboratory atmosphere, care was taken to prepare sample pieces immediately before use. The wire was coiled, using paper to handle it, on a glass rod of approximately ¼ inch diameter. In this arrangement 30 inches of wire formed a coil about 2 inches long. Longer coils were bent horseshoe fashion so that all would fit into wide mouthed 250 cc. Erlenmeyer flasks. Cold (40°F.) milk or cream in 100 cc. portions was introduced into the flasks containing the coils and thin sheets of aluminum foil were placed over the mouths of the flasks and crumpled down tightly over the lips to prevent evaporation and uncontrolled aeration. Heating to 143° F. required 5 to 6 minutes and was effected by placing the flasks into a water bath at 160°F. and agitating the flasks with a circular motion of the hand. They were then transferred to another bath and held at 143°F. for 30 minutes. After

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holding for 15 minutes the milks were agitated gently and after 30 minutes the flasks were transferred to an ice-water bath where they were cooled to 50°F. by agitating for 15 minutes. The copper was removed and the milks were poured into gill bottles and closed with a regular milk bottle cap under which was inserted a square piece of thin aluminum foil to prevent the milk from ever being in contact with the paper cap. The samples thus prepared were stored for 24 and 48 hours at 40°F. and tasted after warming to 80° or 90°F. The samples were always tasted by two judges whose opinions were in good agreement. The following system of scoring was used:

Character of Flavor	*Score*
No off flavor	4.0
Slight off flavor; questionable	3.5
Slight oxidized flavor	3.0
Definite oxidized flavor	2.0
Strong oxidized flavor	1.0
Very strong oxidized flavor	0.0

A given sample was rated by adding the scores of each independent tasting at the two storage periods, and dividing by 4. The scores of the oxidized flavor samples were usually about 1 point lower at 48 hours than at 24 hours. Although this system of grading is not applicable to all flavor defects, it served very well since a more or less "pure" type of oxidized flavor always developed; namely, that induced by metallic copper under comparable conditions. The fat content was determined by the Babcock method.

EXPERIMENTS

Mixed raw certified milk (July 1935) was separated at 95°F. to cream of 32% fat. The cream and skim milk were recombined to form a series of products ranging in fat content from 0.04% (skim) to 8.6%. To 100 cc. portions of each product were added pieces of coiled copper wires of lengths

TABLE I

First experiment: Effect of fat content on the sensitivity of standardized certified milk to copper-induced oxidized flavor

SQ. INCHES COPPER PER 100 CC.	FLAVOR SCORE						
	0.04% fat	0.9% fat	2.9% fat	4.9% fat	5.1% fat*	6.8% fat	8.6% fat
0.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
1.4	4.0	4.0	3.8	3.9	4.0	3.6	3.8
2.7	4.0	4.0	3.6	3.8	2.5	3.3	3.0
5.4	3.8	3.6	3.1	2.8	1.5	2.3	0.8
8.1	3.4	3.3	2.3	1.3	0.8	0.8	0.0

* This sample was the unstandardized stock milk.

corresponding to areas of 1.4, 2.7, 5.4, and 8.1 square inches. The milks containing the copper were then pasteurized and handled according to the

TABLE II

Second experiment: Effect of fat content on the sensitivity to copper-induced oxidized flavor of standardized milk and cream from country cooling station mixed raw milk

SQ. INCHES COPPER PER 100 CC.	FLAVOR SCORE					
	0.025% fat	4.3% fat*	5.0% fat	12.0% fat	20.0% fat	40.0% fat
0.0	4.0	3.9	3.9	3.8	4.0	4.0
0.9	3.8	3.6	4.0	4.0	3.8	4.0
5.4	4.0	1.3	2.0	1.8	0.8	0.3

* This sample was the unstandardized stock milk.

standard procedure described above. The flavor scores of the samples are shown in Table I. The relation between fat content and copper surface required for the induction of oxidized flavor of 3.0 score (estimated by interpolation) is shown graphically in Figure 1. It appears that changes in fat content or the variations in the composition of milk which accompany increase in fat content, significantly affect the sensitivity of the milk. It is to be noted that the stock milk (control) values do not fit into the curve of the standardized products. Apparently separation produced a marked change in the products. In the skim milk of this experiment (0.04% fat) a slight off flavor developed when it was pasteurized with relatively large amounts of copper surface.

A second experiment was made in which mixed raw milk procured from a country cooling station in August, 1935, was separated at 95°F. and products ranging in fat content from 0.025% to 40.0% were prepared. Portions of 100 cc. were pasteurized according to standard procedure with 0.9 and 5.4 square inches of copper surface. The resulting effect on flavor is shown in Table II. The scoring of the high-fat samples on a comparative basis with milk was difficult; but it appears that the sensitivity continues to increase with increase in fat content or the changes accompanying variation in fat content.

A third experiment was made in which mixed raw milk procured from a country cooling station in August, 1935, was separated at 95°F. and

TABLE III

Third experiment: Effect of fat content on the sensitivity to copper-induced oxidized flavor of standardized milk and cream from country cooling station mixed raw milk

SQ. INCHES COPPER PER 100 CC.	FLAVOR SCORE				
	0.03% fat	3.7% fat	18.0% fat	26.5% fat	33.0% fat
0.0	3.8	.....	4.0	4.0	4.0
2.7	3.8	3.5	3.5	3.2	2.7
5.4	3.3	2.0	0.7	1.0	0.7

TABLE IV

Fourth experiment: Effect of fat content on the sensitivity of standardized certified milk and cream to copper-induced oxidized flavor

SAMPLE NO.	FAT CONTENT %	FLAVOR SCORE							
		Square inches copper per 100 cc. of product							
		0.0	1.4	2.7	3.1	5.4	6.8	8.1	10.8
13	0.04	4.0	3.8	3.9	3.9	3.7	3.8	3.7	3.5
12	1.1	4.0	3.9	3.9	3.9	3.8	3.7	3.4	3.3
11	1.8	4.0	3.9	3.8	3.9	3.6	3.6	3.6	3.3
10	2.8	4.0	4.0	3.8	3.8	3.4	3.4	3.3	2.5
1*	3.3	4.0	3.6	3.3	2.6	2.2	2.6	1.7	1.0
2**	3.3	4.0	3.8	3.5	3.3	2.6	3.0	2.2	2.0
9	3.6	4.0	3.9	3.9	3.5	3.3	3.1	3.2	3.1
8	5.4	3.9	3.8	3.5	3.6	3.7	3.2	2.7	1.7
7	7.3	4.0	4.0	3.8	3.3	2.7	2.7	2.3	1.5
6	8.4	4.0	3.8	3.8	3.4	2.7	3.3	3.1	1.2
5	20.0	4.0	4.0	4.0	3.6	3.3	2.3	2.0	1.5
4	26.3	4.0	3.9	3.8	3.3	3.0	2.5	2.3	1.3
3	37.5	3.8	3.9	3.8	3.4	3.5	2.2	1.7	1.5

* This sample was the unstandardized stock milk held cold.

** This sample was the unstandardized stock milk held at 95° F. for 30 minutes.

TABLE V

Fourth experiment: Chemical and bacteriological data on products used

SAMPLE NO.	% FAT	RAW PRODUCTS		PAST. PRODUCTS		% Lecithin
		Microscopic count	Colony count	No copper	10.8 sq. in. copper	
				Colony count	Colony count	
13	0.04	< 14,000	5,600	20	10	0.011
12	1.10	< 14,000	5,800	< 10	10	.....
11	1.80	14,000	7,750	20	30	0.018
10	2.75	< 14,000	5,900	< 10	10	0.017
1	3.30*	56,000	4,050	20	< 10	0.025
2	3.30**	< 14,000	3,200	< 10	20	.....
9	3.55	< 14,000	6,600	< 10	< 10	0.020
8	5.35	< 14,000	6,500	< 10	20	.....
7	7.25	< 14,000	5,050	30	30	0.037
6	8.35	< 14,000	7,900	20	30	.....
5	20.00	28,000	6,850	< 10	30	0.048
4	26.25	< 14,000	4,400	< 10	< 10	.....
3	37.50	14,000	2,650	10	< 10	0.163

* Sample No. 1 was cold raw milk.

** Sample No. 2 was raw milk held at 95° F. for 30 minutes.

products ranging in fat content from 0.03% to 33.0% were prepared. Portions of 100 cc. were pasteurized according to standard procedure with 2.7 and 5.4 square inches of copper surface. The resulting effect on the flavor

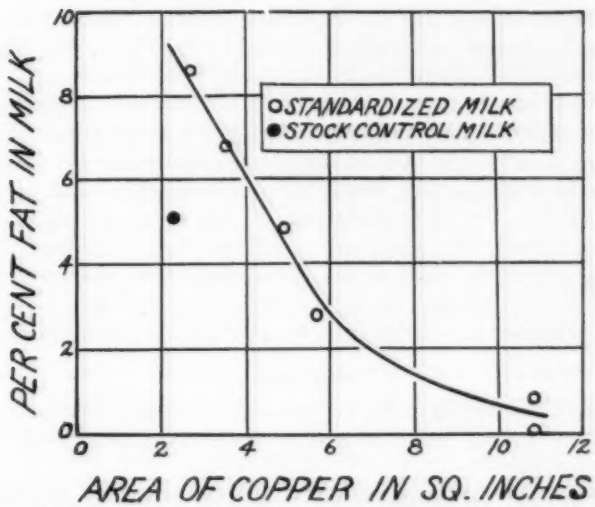

FIG. 1. AREA OF COPPER REQUIRED FOR THE PRODUCTION OF OXIDIZED FLAVOR IN STANDARDIZED MILK (100 CC. SAMPLES).

of the products is shown in Table III. The unstandardized stock milk sample in this experiment was lost.

A fourth experiment was made in October, 1936, in which fresh raw mixed (mostly Holstein) certified milk was separated at 95° F. and a series of products ranging in fat content from 0.04% to 37.5% were prepared from the cream and skim milk. Special care was taken to carry out the whole operation aseptically. All equipment was sterilized with hot water and steam and the skim milk and cream were run into ice packed containers and kept below 50°F. Samples of the original cold milk, the same milk warmed to 95°F. and held until separation was complete, and samples of the standardized products taken the following day when they were opened for laboratory tests, were subjected to bacteriological and chemical examination. Portions of 100 cc. were pasteurized according to standard procedure with 1.4, 2.7, 3.1, 5.4, 6.8, 8.1 and 10.8 square inches of copper surface. Samples of milk pasteurized with 10.8 square inches copper per 100 cc. and of the control pasteurized with no copper were also examined bacteriologically. The raw stock samples were tested for fat content and the lecithin content of five of them was determined by the method of Weise *et al.* (3).

The flavor scores of the samples are shown in Table IV. Chemical and bacteriological data on the samples are summarized in Table V. From the results it appears that the recombined milk was considerably less sensitive to oxidized flavor than the control unseparated milk and that a definite relation again was shown between fat content and sensitivity. It appears also

that separation lowered the phospho-lipoid (lecithin) content of the milk as is shown by comparing sample No. 1 and No. 9 in Table V. Assuming a direct relationship between the fat content of these samples and the phospho-lipoid content then a standardized milk of the same phospho-lipoid content as the control milk would have a higher fat content than the control milk. This suggests that the phospho-lipoid content of the standardized milks may be related to their sensitivity to oxidized flavor and supports the work of Thurston, Brown, and Dustman (4) who concluded that the lecithin content was related to the sensitivity of milk products to oxidized flavor.

The bacteriological examination shows that there was no appreciable growth of micro-organisms during the experiment and minimizes the possibility of decreased sensitivity from the growth of micro-organisms.

CONCLUSIONS

A method was developed for determining the relative sensitivity of milk and cream to oxidized flavor induced by pasteurizing them in the presence of metallic copper.

The sensitivity of standardized milk and cream to copper-induced oxidized flavor appears to be definitely related to the composition of the products as determined by the fat content. A variation of about 1% fat in the range of whole milk was detected by a significant change in the flavor score. Skim milk exposed to large areas of copper surface developed a slight metallic flavor but never an oxidized flavor.

The mechanical separation of milk produced a marked decrease in its sensitivity to copper-induced oxidized flavor as evidenced by tests on milk made by recombining cream and skim milk. Removal of lecithin or related substances by the separator or changes in their distribution between fat and aqueous phase may be responsible for the decreased sensitivity. This suggests a means for reducing the sensitiveness of commercial milk to oxidized flavor.

ACKNOWLEDGEMENT

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THE CHEMICAL ANALYSIS OF BUTTER FOR MOISTURE, SALT, CURD AND FAT

At present there are two generally accepted methods for butter analysis. The older one, advocated by the Association of Official Agricultural Chemists (A. O. A. C.), and used by the Federal Government and States Chemists, has however not been favored by the average creamery operator. A later method suggested by Kohman (Kohman, Edward S., Journal of Ind. and Eng. Chem., Vol. XI, No. 1, p. 36, 1919) because of its greater simplicity, has attained much wider usage in creameries of the country. Changes have been suggested so that now the method is frequently referred to as the "Modified Kohman" method. There is a great lack of agreement among the users of the latter test, both as to equipment and procedure. Because of this, and also because it is so universally used by the industry, the American Dairy Science Association subcommittee on the chemical analysis of butter felt that it should be given primary consideration. There should be available to the creameries and smaller commercial laboratories of the country standard instructions, so that uniformity in results may be expected. This report supersedes a previous report (JOUR. DAIRY SCI., Vol. 13, p. 380, 1930) of this committee.

In the discussion of equipment as well as methods, alternate suggestions are included, so that the laboratory with sufficient funds or with trained personnel may select those which appear most practical. Those alternate suggestions which produce equally satisfactory results will be found in footnotes.

EQUIPMENT AND SUPPLIES

(A) A spatula with a stiff 4″ stainless steel blade for taking and mixing samples.

For larger laboratories:

A mechanical mixer as suggested by D. H. Nelson (JR. OF DAIRY SCIENCE, Vol. XVIII, No. 10, p. 667). It consists of an electric motor having a speed of 1725 r.p.m. and a rating of ¼ horsepower. A chuck similar to that of an electric drill is mounted directly on the shaft. A solid center bit which has had the tip or worm and the two cutters removed is placed in the chuck. When the motor turns the bit is turned in the opposite direction from that when it is used for boring. This motion throws the butter into the bottom of the sample jar which is slipped over the bit. The bit should be preferably of ¾ inch size, and have a solid shaft through the ribs in order to facilitate cleaning. Finally a piece of tin shaped in a half circle is placed on the bench over the bit in order to catch any pieces of butter that may fly off the

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bit when the sample jar is removed. Sample jars should have straight sides and flat bottoms. Their capacity should be 190 per cent of the volume of butter. Temperatures of 53° to 77° F. are satisfactory.

Wilster (Oregon Agricultural Expt. Sta. Bul. 338) designed a stirrer with a vertical specially constructed agitator. The jar of butter is placed on a platform and held in place by a spring while a 1/30 h.p. motor with a variable speed of from 0 to 1725 r.p.m. drives the agitator. The features of the agitator should be such that all parts of the butter are stirred.

(B) A suitable sample jar of non-absorbent material, preferably with a straight side and a tight fitting non-absorbent closure such as a metal screw cap or glass top with a rubber gasket. Four ounce aluminum screw cap jars permit writing identification numbers on the cap.

(C) A polished aluminum beaker having a capacity of 150 ml. and which is at least 3″ tall.

(D) Crucible tongs sufficiently large to handle the aluminum beakers.

(E) A special type balance for the determination of moisture and fat. The balance should have a sensibility reciprocal of not over 15 milligrams. The beams should be so graduated that percentages may be read to the nearest 0.1 per cent if a 10 gram sample of butter is used.

Wilster (Oregon Agr. Expt. Sta. Bul. 338) suggests a modified balance which may be obtained from the Torsion Balance Company, 92 Reade St., New York, N. Y. This balance differs from those usually found in dairy plants, in that it carries an auxiliary 10 gram beam, graduated from 0 to 100 in 10 per cent divisions. Only one 10 gram weight is necessary if this balance is used, provided the cups are of very nearly the same weight.

(F) A 10 gram weight, if the modified balance is used, or a 10 gram and a one gram, or a nine gram weight. For larger laboratories a block of weights sensitive to 0.01 grams is suggested.

(G) A tripod with asbestos mat and a laboratory alcohol lamp or gas flame. Instead of the alcohol lamp or gas flame an electric hot plate with a three heat switch or a high pressure steam oven with a temperature not over 300° F. may be used satisfactorily. For larger laboratories a vacuum oven similar to that used with the Mojonnier tester may be advantageous.

(H) A cooling plate, such as an old flat iron, may be used provided it is located in a dry room and if a glass cover is used on the beaker. A humidor may be converted into a home-made desiccator by placing a drying chemical, such as calcium chloride, in the bottom with a metal platform above, for supporting the cups. The cover may be made air-tight by spreading vaseline around the edges. For faster work a Mojonnier type cooling dessicator or a warming and cooling chamber

as proposed by Wilster (Oregon Agr. Expt. Sta. Bul. 338) may be used. The latter consists of a small closed tank which has several compartments, each closed at the bottom and with a diameter slightly greater than the aluminum cups used. Additional compartments may be provided for sample jars. The water surrounding these compartments is heated by steam for warming the samples or it may be used for cooling by passing cold water through the tank. Since the tank is closed, any danger of water splashing into the samples is avoided.

(I) A[1] suitable fat solvent such as petroleum ether. In order to be satisfactory a solvent should have a low specific gravity, leave no residue upon evaporation, should volatilize rapidly, and be moisture free, since otherwise a small quantity of salt will go into solution. The U. S. Pharmacopoeia requirements for petroleum ether are: specific gravity 0.634 to 0.66 at 77° F.; U.S.P. distillation between 95° to 176° F.

(J) A roll of tissue for wiping triers or spatulae.

(K) A rubber tipped glass stirring rod.

(L) Silver nitrate solution prepared by dissolving 29.064 grams of pure silver nitrate crystals in distilled water and making up to one liter.

(M) A small supply of a 5 per cent potassium chromate solution in distilled water.

(N) A 50 ml. burette with stand or a Nafis automatic type flask and burette.

(O) A 250 ml. volumetric flask.

(P) A 25 ml. pipette.

(Q) A white cup.

(R) A butter trier (not always necessary).

PROCEDURE FOR ANALYZING BUTTER

(A) Sampling.

1.[2] From the churn.

Select a clean thoroughly dry sample jar. With a ladle remove the top layer of butter from a small area, and by use of the spatula, immediately remove 10 to 15 grams of butter from the exposed area. Ten to twelve such samples should be taken from nearly equally spaced areas along the full length of the churning. Care should be exercised that moisture from the walls of the churn does not drop into the sample jar.

[1] High test gasoline and some special low priced cleaning solvents may be used satisfactorily. Solvents with a specific gravity up to .73 and distillation between 300° and 400° F. have been used successfully.

[2] If so desired, at least five trierfuls of butter may be taken at right angles to the roll of butter. All butter excepting that which adheres to the back of the trier should be transferred to the sample jar. The trier should be wiped before taking each plug.

2. Sampling a single large box or tub.

A single trierful should be taken by boring diagonally from the top to the bottom. About an inch of butter from each end of the trier should be eliminated. At least two packages from a churning should be sampled, or three if there are more than 20 packages in the churning. If but one package were sampled three trierfuls rather than one as suggested above are to be taken. One of these should be removed from the center and the other two, opposite one another, about half-way between the center and the outside rim.

If the butter has been properly worked, it may be transferred from the trier to the sample jar by use of a spatula. Moisture adhering to the back of the trier is negligible unless the butter is improperly worked, in which event the sample would not be representative and results should be regarded as approximate only.

3.[3] Sampling a one pound print.

The print should be quartered lengthwise and then all quarters cut transversely in half. Two diagonally opposite eighths are used for the sample.

(B) Preparing the Sample for Analysis.

Samples for analysis should be softened by placing in a water bath at a temperature of 90° to 95° F. (depending upon the consistency of the butter). The sample should be the same color as the hard butter and be only soft enough so that it will not retain its shape when raised to a point by the flat side of a spatula. Particular care should be taken to keep the sample jar tightly sealed at all times except when the butter is being stirred or when the sample is weighed. The method selected for mixing depends upon the number of samples to be tested and the size of the laboratory. The following methods are suggested:

1. A four inch stiff bladed spatula. Extreme care is necessary to see that the mixing is complete and that all butter is removed from the corners of the container. A consistency like that of mayonnaise is desirable. The color of the mixed sample should be uniform throughout.
2. Motor driven mixers as proposed by Nelson (JOUR. OF DAIRY SCIENCE, Vol. XVIII, No. 10, p. 667) or Wilster (Oregon Agr. Expt. Sta. Bul. 338). The operator should assure himself that mixing is complete so that the sample is consistent throughout (at least three minutes for the Nelson mixer).

[3] Another acceptable method for well worked butter would be to cut the print in two halves and then a ¼″ to ¾″ slab should be removed from one of the freshly cut surfaces and transferred to the sample jar.

(C)[4] Weighing the Prepared Sample.

When more than one sample is to be tested, the scale first should be balanced. This is done so that the operator may check it before weighing back his cups after the moisture evaporation or fat removal. Thus he is assured of satisfactory weighings. If a Torsion balance is used, the riders on the graduated beams should be at the extreme left. If the large tare rider is on an ungraduated beam it is usually placed at the extreme right of the beam. The beaker should be weighed by placing it on the right-hand pan and its weight recorded. The beaker used should be perfectly clean and dry and of constant weight. It should be weighed cold. Under ordinary conditions exactly 10 grams of the prepared sample of butter should be weighed into the beaker as quickly as possible, and should be placed on the bottom of the dish if possible.

(D) Evaporation of the Moisture.

The beaker is transferred to an electric hot plate, an alcohol lamp, gas flame or pressure oven to evaporate the moisture. If an open flame is used it is advisable to use an asbestos screen or pad to prevent the deposit of carbon on the beaker. The evaporating temperature should not exceed 300° F. since higher temperatures tend to produce spattering. The sample should be agitated frequently so that the formation of a casein scum is prevented. Evaporation should be continued until the color is a golden brown or until no fresh foam bubbles are formed.

(E) Cooling the Sample.

The dried sample should be weighed at the same temperature as the original weighing. Cooling should be so carried out that no moisture is reabsorbed by the sample. Neither should free moisture cling to the beaker and, therefore, cooling the sample by holding the beaker in cold water is inadvisable except by a highly trained operator and only when highly polished beakers are used.

The most satisfactory methods of cooling are those suggested under the list of cooling equipment.

(F) Determining the Moisture Content.

The perfectly dry, cool beaker is placed on the right hand pan of the previously balanced scale and the tare weights adjusted. If a 10

[4] If larger laboratories prefer, the following method may be used: Approximately ten grams of the prepared sample of butter are placed quickly in the cup or beaker. A watch glass or metal disk should be placed on the beaker as quickly as the butter is delivered into it, so that there will be no evaporation while the exact weight is obtained. When this procedure is followed, a block of weights sensitive to 0.01 gram is necessary. To obtain the amount of butter, the weight of the empty beaker is subtracted from the combined weight of the beaker and butter. (JOURNAL OF DAIRY SCIENCE, Vol. XVI, p. 303.)

gram sample of butter was used, the per cent moisture may be read directly off the graduated beams on the Torsion type scale.

(G) Determining the Fat Content.

1. After the per cent moisture has been determined the sample should be warmed and approximately 100 ml. of solvent added. The mixture should be stirred with a rubber tipped glass or steel rod. The sample should be allowed to stand not less than four minutes, after which the solvent should be poured off slowly until only a few drops remain. Pouring off the solvent without loss of the settlings may be facilitated by setting the beaker at an angle with the pouring lip down during the settling period. The process should be repeated using not over 100 ml. solvent. After the second decantation, the remaining solvent should be evaporated by low heat on the electric hot plate, or by setting on a steam pipe. High temperatures with some solvents result in spattering. When properly dried no solvent odor should be noticeable and the salt and curd remaining in the beaker should be powdery.

2.[5] Weighing the Sample.

After the solvent has been evaporated completely the beaker is cooled, as for the moisture determination. It then is placed on the right-hand pan of the scale, and the amount of residue (curd and salt) remaining in the beaker is determined. A one gram weight may be substituted for the ten gram weight used for weighing the sample, or a nine gram weight may be placed with the cup on the right hand pan, and the ten gram weight retained on the left hand pan. The sliding weights on the graduated beams are moved so the scale is balanced. The readings on the graduated beams (expressed in per cent) when subtracted from ten gives the per cent salt and curd. The per cent of salt and curd plus the per cent moisture, subtracted from one hundred then gives the per cent fat.

By using the scale proposed by Wilster the readings will be simplified.

(H)[6] Determining the Salt Content of Butter.

1. The salt test may be made directly on the residue remaining from the fat test. If a ten gram sample of butter was used the resi-

[5] Where other than a ten gram sample of butter is used the per cent of fat is calculated as follows:

$$\frac{\text{Weight before extraction—Weight after extraction}}{\text{Weight of butter used}} \times 100 = \text{per cent fat.}$$

[6] If so desired, the entire 250 ml. sample may be titrated. Five or six drops of potassium chromate solution are used. One ml. of the silver nitrate solution is equivalent to one-tenth per cent salt.

Slightly greater accuracy may also be attained by use of a silver nitrate solution of one-half the concentration proposed. In that case two ml. of silver nitrate solution are equivalent to one per cent salt.

due is rinsed from the beaker and made up exactly to 250 ml. with warm chlorine free water. Twenty-five ml. of the solution are placed in a white cup and two or three drops of potassium chromate solution added. Silver nitrate solution then is added until a flesh or light orange color develops. One ml. of the silver nitrate solution is equivalent to one per cent salt.

2. If the salt test is made directly upon a ten gram sample of butter, enough warm chlorine free water should be added so that the bottom of the fat column will be at the 250 ml. mark on the flask. Twenty-five ml. of the fat free solution is then used for titration as described above.

(I) Determining the Curd Content of Butter.

The curd content is determined by difference, which means that the per cent salt may be subtracted from the combined salt and curd percentage if the Modified Kohman test has been used.

(J) The accuracy of the method of analysis described does not permit results beyond the nearest one-tenth per cent.

SUBCOMMITTEE ON BUTTER ANALYSIS

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NUTRITIVE VALUE OF CHOCOLATE FLAVORED MILK

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INTRODUCTION

The fact that some dairymen are advertising chocolate milk as a real health food for children and for convalescents and that schools rank second as chocolate milk sales outlets (1) brings up the old question of the nutritive value of cocoa and the question of whether cocoa should be included in children's dietaries. Most of the reported experimental work on this problem is controversial. The extensive use of cocoa has been criticized because it contains theobromine and, to a lesser extent, caffeine. However, pathologists generally agree that the objection to the use of chocolate milk because of the theobromine content is not fundamental. Another objection raised to chocolate milk is its sugar content which is considerably higher than that of whole milk. Although most investigators concede that the tannic acid content in cocoa is too small to be of any significance, they disregard the high content (2.35 to 5.9 per cent) of cacao red which resembles tannin in many of its properties.

Cocoa and chocolate are made from the nibs of cacao-beans. The cacao-bean is the seed of the cacao-tree, and its component parts are the shell, nib and germ. Chocolate is the solid or plastic mass obtained by grinding the roasted or dried nibs; while cocoa, or powdered cocoa, is chocolate deprived of a portion of its fat and pulverized. There are U. S. Federal definitions and standards for cacao products (2). Space does not permit to give the standards in detail; however, it should be mentioned that they specify in general the minimum fat content and the maximum ash and crude fiber content of cacao products. The composition of commercial cocoas varies considerably according to the composition f the cacao-bean used, the extent to which the fat has been removed, and the method of manufacture. The following figures taken from Whymper's "Cocoa and Chocolate" (3) give the main components of commercial cocoa powder.

Cocoa is more likely to be used in milk for its flavor than for its food value. As used in making ordinary chocolate milk, the food value of cocoa is negligible. However, there is a possibility that the small amount of cocoa added to milk may have a marked effect on the digestibility of the milk solids. Whymper (3) states that tannin decreases the solubility of milk solids. Neumann (4), (5) studied the digestibility of cocoa, using himself

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	PER CENT
Moisture	2.25 to 5
Ash, American Process	3 " 5
Dutch Process	5 " 11
Fat	22 " 35
Extractive soluble in water	13.5 " 18.5
Theobromine	0.7 " 2.7
Starch	2 " 11
Fiber	2.5 " 6.5
Proteins	10 " 17
Oxalic acid	0.4 " 0.65
Sucrose	Trace
Cacao red	2.35 " 5.9

as a subject for 86 days, and reports that the addition of cocoa to other articles of food seems to reduce the total amount of nitrogen absorbed. He also found that the amount of fat present in the cocoa affects the absorption of nitrogen, a reduction in fat lowering the assimilation of nitrogen.

OBJECT OF STUDY

In view of these reports, this study was undertaken with the hope of demonstrating by animal feeding experiments whether the addition of cocoa to milk changes the nutritive value of the milk. Also, it was hoped to secure some data which might aid health officials in setting up standards for chocolate milk. The authors are aware of only one instance where such standards have been set up. The Baltimore City Health Department (6) requires a minimum fat content of 2.5 per cent and the permissible maximum cocoa and added sugar are 5 and 6 per cents, respectively. Because of the lack of information on the nutritive value of chocolate milk, it was necessary to depend upon trade practice and consumers' preference when formulating these standards.

EXPERIMENTAL

The general plan was to feed one group of albino rats whole milk and other groups whole milk to which various percentages of cocoa had been added. Both control and the chocolate milk diets were supplemented with cane sugar and with iron, copper and manganese, according to the procedure reported by Elvehjem *et al.* (7). These investigators have made studies which suggest that the rate of growth of male rats on mineralized milk is an excellent measure of changes in the nutritive value of that milk.

The cocoa used throughout this experiment is a commercial product made by the Dutch Process with the following composition.

CONSTITUENT	PER CENT
Moisture	3.00
Ash	7.11
Nitrogen	3.74
Ether soluble material	20.64
Crude fiber	5.25
N. F. E.	40.63

In the first experiments pasteurized fluid milk (Approx. 3.8% butterfat) was fed, while in the later experiments whole milk powder was fed. Details in the experimental procedure are given under each separate experiment.

PRELIMINARY EXPERIMENT

Since no information was available as to whether rats would drink milk containing varying percentages of cocoa, a preliminary experiment was conducted to determine the approximate maximum amount of cocoa that can be added to milk without retarding the rate of growth. Three animals each were placed on a whole milk and on a one per cent chocolate milk diet and one animal each was placed on chocolate milk diets containing respectively 10, 20, and 30 per cent cocoa powder. Fresh, pasteurized, mineralized milk to which seven per cent cane sugar had been added was used for all the diets. The milk was fed *at libitum*. It was found that the daily consumption of chocolate milk by rats decreased as the percentage of cocoa was increased above one per cent. For example the average daily feed consumption during th first week of the experiment was as follows:

No cocoa	35 grams
1% "	35 "
10% "	17 "
20% "	10 "
30% "	7 "

The animals receiving the 30 per cent and 20 per cent chocolate milk died at the end of one week and six weeks, respectively. The animal receiving 10 per cent cocoa in the milk was taken off the experiment after 12 weeks with practically no gain in weight. Animals on the whole milk diet and the one per cent cocoa diet were kept on experiment for 30 weeks, with no significant difference in rate of growth. At the end of the experiment all six animals weighed approximately 400 grams and were extremely fat. The results of this preliminary experiment indicate that rats will make normal growth gains on whole milk and on chocolate milk containing 1 per cent cocoa, but show poor growth and definite injury on milk containing 10 per cent cocoa or more.

FEEDING FLUID CHOCOLATE MILK AD LIBITUM

The purpose of this experiment was to continue the study on the maximum amount of cocoa which may be added to milk without retarding the rate of growth. Twelve male rats, weighing approximately 43 grams each,

TABLE I

Summary of results for first six weeks of experiment

	RATIONS			
	Control	4% cocoa	7% cocoa	10% cocoa
	gm.	*gm.*	*gm.*	*gm.*
Average daily gain	3.45	2.07	0.85	0.04
Average daily feed consumption	53.2	37.3	23.5	16.7
Average daily total solids intake	9.89	8.22	5.75	4.5
Average daily cocoa intake	0	1.49	1.64	1.67

were used in this experiment. The animals were divided into three groups of four rats each. Each group received one of the following four diets: Whole milk without cocoa, and whole milk to which 4, 7, and 10 per cent cocoa was added. The rats were placed in individual cages, which were equipped with Fisher porcelain feed cups and water bottles. All of the

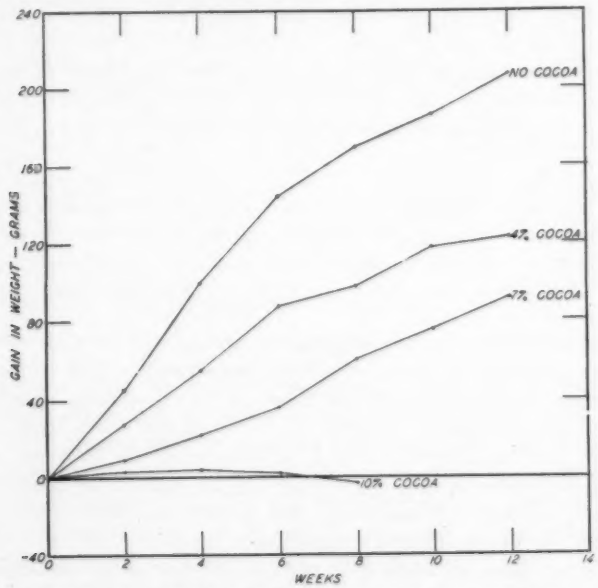

FIG. 1. COMPARATIVE GROWTH OF RATS FED AD LIBITUM MILK CONTAINING VARYING PERCENTAGES OF COCOA.

PERCENTAGES OF COCOA.

No. 1—Received no cocoa, weight 224 grams.
No. 2—Received 4 per cent cocoa, weight 185 grams.
No. 3—Received 7 per cent cocoa, weight 102 grams.
No. 4—Received 10 per cent cocoa, weight 51 grams.

diets were mineralized and contained seven per cent cane sugar, and were fed ad libitum.

The experiment lasted for 12 weeks, and the results obtained are given in Table I, and Figures 1 and 2. Data in Table I are limited to the first six weeks of the experiment, because two rats on the 10 per cent cocoa ration and one rat on the seven per cent cocoa ration died after seven weeks. The other animals made the following average total gains during the experimental period: control, 208 gms; 4 per cent cocoa, 120 gms; 7 per cent cocoa, 92 gms; and 10 per cent cocoa, no gain. Their rates of growth are shown in Figure 1. Table I shows that when chocolate milk varying in cocoa content from 4 to 10 per cent is fed *ad libitum* the average daily gain in weight decreases as the percentage of cocoa in the diet increases. Figure 1 shows graphically these marked differences in weight. An idea of the physical condition of the animals may be gained from Figure 2. The rats had been on experiment for seven weeks at the time the picture was taken. The photograph shows the marked differences in weight and in the appearance of the coats of the animals.

Table I also shows that the daily chocolate milk intake decreases as the percentage of cocoa is increased in the diet. This no doubt is mostly responsible for the inferior growth when cocoa was fed. However it does not seem to account for all of the observed decreases in growth. It should also be noted in the table that the average daily cocoa intake was practically the same for the three concentrations of cocoa. This will be referred to in the discussion of the data.

All animals were autopsied at the close of the experiment or after they had died. In all of the animals the lungs, liver, spleen and the kidneys appeared to be normal. However, the rats receiving the seven, and ten per cent cocoa diets had large masses of what appeared to be undigested cocoa in the ceca. In one case it appeared to be obstructing the whole tract. Furthermore, the feces in the intestinal tract were hard. Gas was found in the stomach, intestinal tract, and cecum. These abnormalities were more pronounced as the cocoa was increased from seven to ten per cent. One rat on the ten per cent cocoa diet showed a possible petechial hemorrhage in the gastric mucosa.

FEEDING COCOA WITH WHOLE MILK POWDER

In this study the cocoa was added to the whole milk powder instead of to fluid milk as in the previous experiments. This feeding procedure has

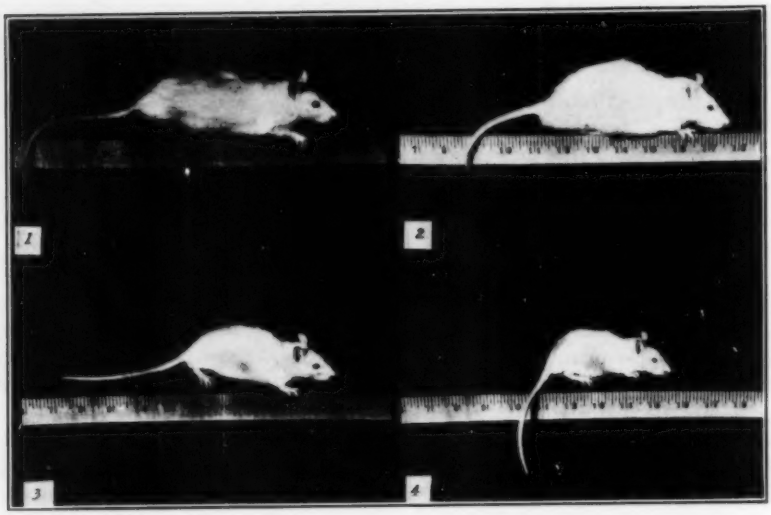

FIG. 2. PHOTOGRAPH OF RATS FED AD LIBITUM MILK CONTAINING DIFFERENT PERCENTAGES OF COCOA.

TABLE II
Formulae for rat rations

INGREDIENTS	RATION NO.			
	I Per cent	II Per cent	III Per cent	IV Per cent
Cocoa	0	5.0	11.9	17.8
Whole Milk Powder	62.6	59.4	54.5	50.6
Cane Sugar	37.4	35.6	33.6	31.6
Cocoa on Fluid Milk Basis	0	1	2.5	4

the advantage that enough feed can be mixed at one time for the entire experiment. Another advantage for the dried milk plus cocoa is that the cocoa does not settle out as in fluid milk, thus making possible a closer check on the daily consumption of cocoa.

Two experiments were conducted with the dehydrated diet, using a total of forty-eight young rats as subjects. In the first experiment 12 males and 12 females were used, while in the second experiment 8 males and 16 females were used. In each experiment 24 young rats were divided into six groups. Each group of four animals consisted of litter mates of the same sex, and as nearly as possible of the same weight. Up to the time of being placed on the experiment, the rats had received the stock ration of the breeding colony.

Four diets were compounded as shown in Table II, and fed one to each rat in the groups of four individuals, thus there being in each experiment

TABLE III

Average daily gain in weight and daily feed consumption during the experiments

RATION	GAIN IN WEIGHT	GAIN OR LOSS OVER CONTROL	BASIC RATION INTAKE	COCOA INTAKE
	gm.	*gm.*	*gm.*	*gm.*
		Experiment No. 1*		
No cocoa	2.21		6.80	0.00
1% cocoa	2.15	−.06	6.80	0.36
2.5% cocoa	2.02	−.19	6.80	0.92
4% cocoa	1.86	−.35	6.80	1.49
		Experiment No. 2**		
No cocoa	1.85		8.57	0.00
1% cocoa	1.90	+.05	8.57	0.46
2.5% cocoa	1.74	−.11	8.57	1.16
4% cocoa	1.49	−.36	8.57	1.86

* Experiment No. 1 lasted for six weeks and each datum is an average value for six animals, three males and three females.

** Experiment No. 2 lasted for nine weeks and each datum is an average value for six animals, four females and two males, except for the one male group in which the rat on the 4% cocoa ration died during the fifth week apparently from some respiratory trouble.

six individuals on each of the four treatments. The rats were fed in accord with the principle of paired feeding, however, in this case quadruplets instead of pairs. The four diets were compounded to contain none, one, two and one-half, and four per cent of cocoa respectively and seven per cent cane sugar, on a fluid milk basis. Each rat in a group of four received the same amount of milk powder and cane sugar but had a different cocoa intake. In other words, the only variable in the ration was the percentage of cocoa which was added as an accessory food. The quantity of food given to each group of four rats was determined by the quantity consumed by the individual eating the least within the group. In most instances, the four per cent cocoa diet determined the food intake in all groups.

The rats were individually caged and placed on the experimental diets shortly after weaning. The first experiment was continued for six weeks and the second for nine weeks. The animals were fed once each day and the feed was weighed daily to determine the amount consumed, care being taken to prevent losses. The food was weighed into a Fisher porcelain feed cup of approximately 75 c.c. capacity, which was set in a metal cup and held in place by a metal cover. This arrangement reduced the average spillage per rat during six weeks to approximately two grams. The iron, copper, and manganese were fed daily in amounts as recommended by Elvehjem *et al.* (7), and were added to the milk powder. The rats had water before them all of the time.

The rats were weighed weekly. Bacterial flora studies were made during the latter part of the experiments. At the close of both experiments some of the rats on each diet were autopsied.

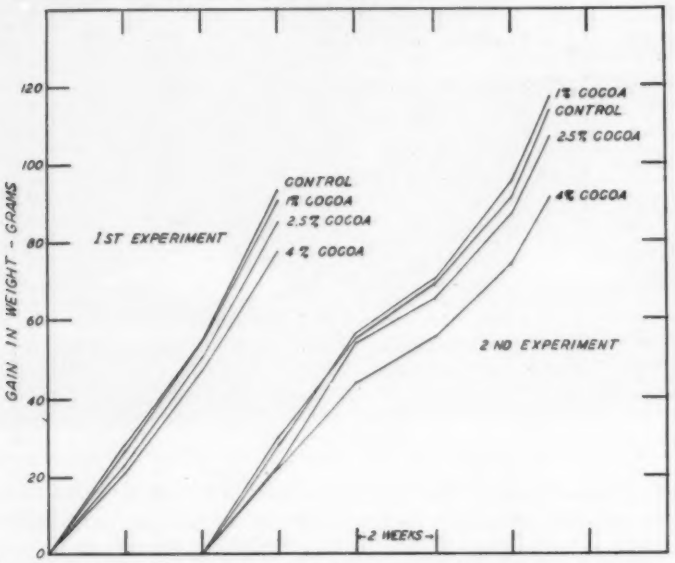

FIG. 3. COMPARATIVE GROWTH OF RATS FED EQUAL AMOUNTS OF THE BASIC RATION BUT VARYING AMOUNTS OF COCOA.

The essential data are given in Table III and Figure 3. Since the rate of growth varies with the sex of the animals, the data were analyzed separately. It was found, as expected, that the females grew more slowly than the males. It was also found that the trend in the rate of growth, both for the control rats and for those receiving varying percentages of cocoa, was practically the same for both sexes. Since the sexes were equally distributed for all rations, the data are presented as averages for male and female.

The growth curves in Figure 3 and the data in Table III for the first experiment show a progressive decrease in rate of growth as the percentage of cocoa is increased up to four per cent. However, decrease in rate of growth is probably not significant until the amount of cocoa is increased to four per cent.

The second experiment was started with two males and four females on each diet. During the fifth week one male on the four per cent cocoa diet died, presumably from a respiratory trouble, thus reducing the male group to one animal on the four per cent diet during the latter part of the experiment. Both male and females grew a little more slowly in the second ex-

periment than they did in the first. The only known difference in the diets of the two experiments was that the whole milk powder was obtained from a different lot in the second experiment. The results obtained in the second experiment are similar to those obtained in the first; namely, that the cocoa must be increased to four per cent before a significant decrease in the rate of growth is obtained. The one per cent cocoa group made slightly better gains than the control, while in the first experiment the reverse was true. However, the differences between the control, one per cent, and 2.5 per cent cocoa diets are not great enough to be of any significance. The autopsies showed no pathological condition in any of the experimental animals.

EFFECT OF COCOA ON THE INTESTINAL FLORA OF ALBINO RATS

A study was made of the intestinal flora of three rats on each of the following diets: Powdered whole milk, powdered whole milk plus 4 per cent cocoa, fluid whole milk, and fluid whole milk plus 1 per cent cocoa. All of the diets were mineralized and seven per cent cane sugar was added. These animals had been on the powdered milk diet for eight weeks and on the fluid milk diet for 30 weeks, when this study was made. A detailed description of the feeding procedure has already been given. Serial dilutions of the feces were plated out on nutrient agar, aerobic and anaerobic, MacConkey's agar, and tomato agar in an atmosphere of carbon dioxide. Egg meat tubes were used to determine the degree of hydrogen sulphide production and putrefaction.

The results obtained indicate that there was no significant difference in the intestinal flora of the rats on the different milk diets. From the differential plate counts it was found that aciduric bacteria strongly predominated over the *Escherichia coli,* anaerobes, and other fecal bacteria. The data obtained from the egg meat tubes showed the presence of putrefactive bacteria in all of the rats. It is probable that the putrefactive bacteria present were *Clostridium welchii,* a common intestinal anaerobe. It is well known that diet has a marked influence on the type of bacteria present in the intestinal tract. As all of the experimental rats were on straight milk diets, it was to be expected that they would all have a similar intestinal flora. Although some of the animals received one and four per cent cocoa in the milk, this amount of cocoa is not enough to make a marked change in the composition of the milk diet, in-so-far as its influence upon the intestinal flora is concerned.

pH determinations were made on the feces of three animals on whole milk diet and three animals on whole milk diet to which one per cent cocoa was added. It was found that the average pH for the cocoa-free diet was 7.32 and for the one per cent cocoa diet was 7.40. It is evident from these fecal pH values that the addition of one per cent cocoa to a straight milk diet has no significant effect on the fecal pH.

DISCUSSION

When chocolate milk was fed *ad libitum* to rats, the daily consumption decreased as the percentage of cocoa was increased above one per cent. It was noted that when feeding *ad libitum* chocolate milk which contained four, seven, and ten per cent cocoa, the daily cocoa intake was nearly the same, being 1.5, 1.6 and 1.7 grams, respectively. In the controlled feeding experiments in which all animals within a group received equal quantities of the basic ration but varying amounts of cocoa, the diet containing the highest percentage of cocoa determined the food intake for all the animals in the group. These results indicate that cocoa limits the consumption of chocolate milk by rats. Two possible reasons for this are first, the cocoa may decrease the palatability of the milk; second, the cocoa may be toxic to rats.

This study has shown that there is a narrow range of cocoa tolerance in rats. One per cent cocoa in milk had no noticeable effect, two and one-half per cent cocoa had a questionable effect, while four per cent cocoa retarded the growth of rats. It is difficult from our present results to determine what specific factors may be responsible for this retardation in growth. The feces in the intestinal tract were very hard when the concentration of cocoa was increased to seven or ten per cent in the diet. Therefore, the effect of cocoa may be mostly a physical one in that the indigestible cocoa fiber tends to block the intestinal tract. Neumann (4) has shown that the protein in cocoa is more digestible in the presence of larger quantities of cacao fat. This suggests that chocolate milk made from whole milk may be more easily digested than that made from skimmilk.

Further study is being made to determine what specific factors are responsible for the observed retardation in growth when four per cent cocoa is added to milk.

SUMMARY AND CONCLUSIONS

The effect of the addition of varying percentages of cocoa to mineralized whole milk was studied by means of growth experiments on a total of 72 albino rats. When fluid chocolate milk containing more than one per cent of cocoa was fed *ad libitum*, the rate of consumption decreased as the percentage of cocoa increased.

When cocoa was added to whole milk powder and the amount fed was controlled, the one per cent cocoa diet was equal to the whole milk diet; the two and one-half per cent cocoa diet gave a questionable retardation rate of growth; and the four per cent cocoa diet definitely retarded growth.

When the rats received the seven and ten per cent cocoa diets, the feces in the intestinal tract were very hard and there was a greater accumulation of food material in the ceca than was the case in the control group.

Studies of intestinal flora showed no distinctive changes for the whole milk, one, and four per cent cocoa diets. The addition of one per cent cocoa to a straight milk diet had no significant effect on the fecal pH.

Since these experiments were conducted with laboratory animals only, no direct application to human nutrition can be made. Assuming, however, that these results may have some application to human nutrition, we may conclude that the cocoa in average commercial chocolate milk which contains a trifle over one per cent cocoa does no harm nor does it enhance the nutritional value of the milk.

The authors wish to express their indebtedness to Dr. H. Rakieten of the Department of Bacteriology and Physiology for performing the autopsies and also to Mr. W. B. Esselen, Jr., of the Nutritional Laboratory, for making the intestinal flora studies.

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THE RELATIONSHIP BETWEEN TEMPERATURE AND OVERRUN IN THE WHIPPING OF ICE CREAM MIXES

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It is the purpose of this paper to present data showing that the whipping capacity of various ice cream mixes can be expressed by straight line equations of the form

$$\% \text{ Overrun} = At + B$$

where t is the temperature in the freezer and A and B are constants, subject to the qualification that each mix, as it approaches complete melting, reaches a maximum possible overrun for that mix; and subject possibly to a further limiting figure, capable as yet of but approximate evaluation, which may describe the stability of the whip to continued beating in the freezer. The whipping capacity of a mix will be slightly different in different freezers but the equation, plus a statement of maximum overrun and stability, will describe the whipping properties of each mix under given freezing conditions. It is believed that this is true of all ice cream mixes of usual composition and treatment, since it held for all of the considerable number of mixes studied.

The relationship of the above information to the usual manufacturing procedure for ice cream is discussed.

INTRODUCTION

The capacity of an ice cream mix to incorporate air in the freezer is one of its most important properties, particularly from the point of view of economy in manufacture. The degree of overrun which is obtainable in a mix is dependent in varying degree upon every variable that enters into the process of manufacture, and since, up to the present time, there has been no way to evaluate adequately the whipping capacity of a mix, information concerning the effects of these variables is in a chaotic state. Work was therefore undertaken to find out if any simple relationship existed between the whipping capacity of a mix and the temperature of the ice cream in the freezer.

Note:—A check of the homogenizer gauge after this paper was in press showed that the actual homogenization pressures must have been lower than recorded. A repetition of a portion of the work showed that the general principles of the paper are correct. As might be expected, the mixes prepared at higher pressures were somewhat more stable than those recorded herein.

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EXPERIMENTAL

The best way to determine whether or not there is any relationship between whipping capacity and temperature in an ice cream mix would be to freeze to a definite temperature, whip to either maximum or constant overrun, and compare the figures with the data obtained at different temperatures with other portions of the same mix. To this end the 20-quart Miller brine freezer (dasher speed 180-R. P. M.) was connected through a pump and suitable valves to two brine tanks. The brine in one tank was maintained at a temperature of – 17.8° C. (0° F.) or slightly below, and was used to freeze the mix down to the desired temperatures. The brine from the other tank, which was usually brought to a temperature approximately 3° C. below that desired in the freezer could then be circulated through the freezer to maintain a constant temperature. A predetermined temperature could not be obtained exactly, but for the purpose of the work any approximate temperature was suitable if it could be maintained. In the course of the work the temperature of the ice cream was measured in the overrun cup. Work carried out a number of years ago comparing such readings with those of a thermocouple within the freezer indicated that, as long as the cold brine did not cause supercooling within the freezer, this method was reliable.

When mixes were frozen in this way it soon became apparent that a definite maximum overrun was attained in the freezer shortly after constant temperature was reached, but that this higher value could not usually be held longer than from 2 to 8 minutes, in spite of the fact that temperatures could be maintained constant practically at will. The ability of a mix to withstand the action of the beaters while at its maximum overrun is apparently an important property of each mix, as will be shown later.

It should be mentioned that Thomas Hall[1] carried out a similar series of experiments upon a mix of 38% total solids and 10.75% butter-fat, but his mix was apparently very stable, for he obtained constant overrun with continued whipping at constant temperature. He also noted that 100% overrun was obtained for this mix at a constant temperature of – 3.25° C. even though this temperature was reached in different ways. From this he concluded that temperature and overrun were interrelated, but unfortunately he appears not to have carried the work further.

In plotting overrun against temperature for our data obtained in the above manner, it became evident that a simple straight line relationship existed between overrun and temperature if the temperature was not too high. If a certain temperature, characteristic for each mix, was exceeded, overrun began to fall off rapidly. Some of Hall's data, when re-plotted in this manner also gave straight lines. If temperatures are permitted to go too low, there seems to be a departure from the straight line relationship, the

[1] Thomas Hall, Ice Cream Trade Jour. *20*, no. 10, p. 51, 1924.

overruns being higher than expected. These are temperatures where overruns are about 40% or less and are lower than would usually be encountered in commercial practice. This phase of the subject has not yet been investigated thoroughly.

In Table 1, and Figure 1, data and curves are given showing the relationships encountered between overrun and temperature for mixes of varying composition, gelatin content, and homogenization pressure. The data are not presented as a study of these factors in relation to overrun, although some interesting relationships are apparent, but rather to show that mixes of wide variety exhibit a straight line overrun-temperature relationship. This relationship can be expressed by the equation for a line as follows:

$$\% \text{ Overrun} = At + B$$

where t is the temperature and A and B are constants. The constant A indicates the rate of overrun increase with rise in temperature and B locates the line with relation to the coordinates. In general it could be said that the greater these constants, the greater the overrun obtainable at a given temperature, but since a certain overrun cannot be exceeded in each mix this equation is not a complete description of whipping capacity unless limited by the statement of the maximum possible overrun that can be attained in the mix.

The question now arises as to the relation of this information to overrun data obtained in normal freezings in this same brine freezer. With the fact in mind that the usual method of freezing ice cream is to freeze down to a certain point in the freezer and then turn off the brine and whip till the desired overrun is reached, several portions of each of the mixes reported above were frozen to different temperatures, the brine was shut off and the mixes were whipped for a considerable period. Temperature, overrun and time were recorded throughout this process. It was soon evident that in every case the temperature-overrun equilibrium line was reached in from 12 to 14 minutes and that this straight line was followed for a period as the mix continued to warm up. This period was sometimes brief and sometimes long. Then with continued beating the whip broke and the overruns became lower than called for by the equation of the equilibrium line. This is shown by the data plotted in Fig. 2, obtained from the 12% butter-fat mix made up without gelatin, one of the most stable mixes.

In plot A the straight line is given. In the other plots it is dotted in for reference. Plot B is for a freezing in which the brine was turned off at a comparatively high temperature. It is seen that the overrun-temperature curve reaches the equilibrium line at about its mid-point and follows it to its maximum. In plot C the mix is frozen to a lower temperature, reaching the line at about 78% overrun and following it to nearly 120% overrun, when the overrun drops with further increase in temperature. In Plot D

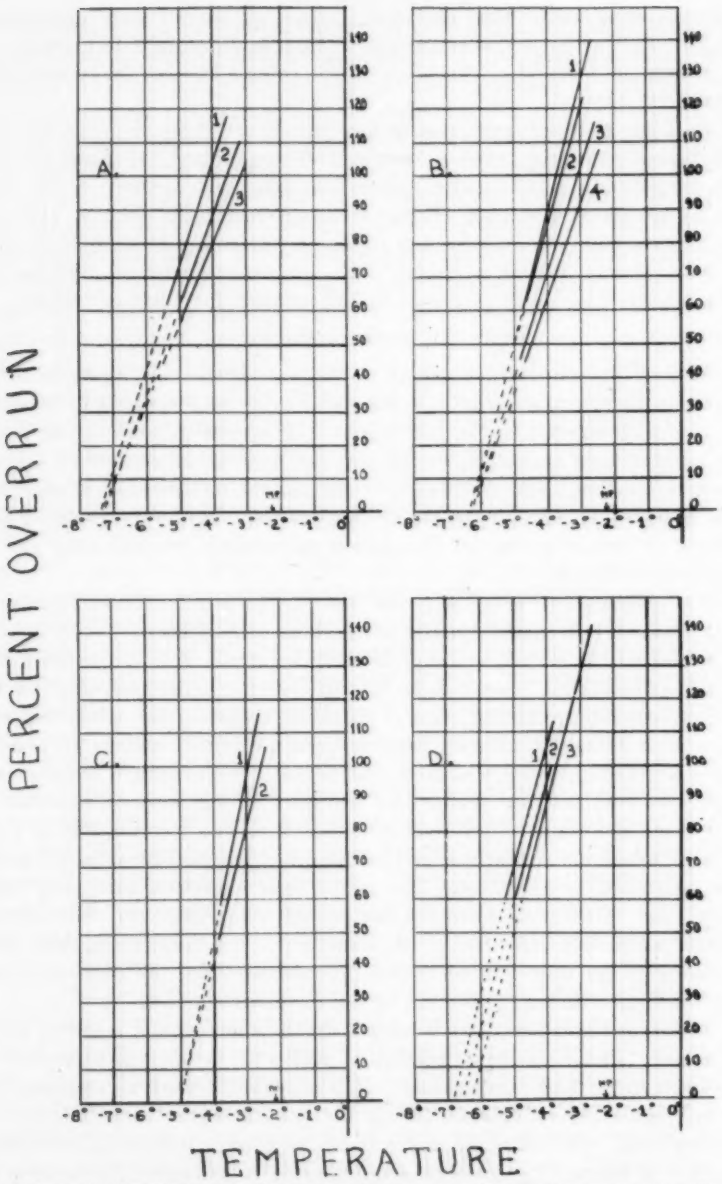

FIGURE 1. RELATIONSHIPS BETWEEN OVERRUN AND TEMPERATURE FOR ICE CREAM MIXES OF VARYING COMPOSITION, GELATIN CONTENT AND HOMOGENIZATION PRESSURE.

TABLE 1

Relationships between overrun and temperature for ice cream mixes of varying composition, gelatin content and homogenization pressure

A. Mixes of 8% butter-fat, 10% milk-solids-not-fat and 14% sugar, 2500 lb. homogenization pressure.

1.—No gelatin		2.—0.3% gelatin		3.—0.5% gelatin	
Overrun	Temp. °C.	Overrun	Temp. °C.	Overrun	Temp. °C.
110	−3.80	98	−3.50	97	−3.20
99	−4.10	93	−3.80	90	−3.50
91	−4.50	85	−4.10	82	−3.90
96	−4.60	80	−4.40	70	−4.50
75	−5.00	76	−4.50	60	−4.90
66	−5.20	70	−4.60		
% Overrun = 30.7t + 227		% Overrun = 27.0t + 195		% Overrun = 24.2t + 178	
Max O. R. 115%		Max. O. R. 108%		Max. O. R. 105%	

B. Mixes of 12% butter-fat, 10% milk-solids-not-fat and 14% sugar, 2500 lb. homogenization pressure.

1.—No gelatin		2.—0.1% gelatin		3.—0.3% gelatin		4.—0.5% gelatin	
% Overrun	Temp. °C.	% Overrun	Temp. °C.	% Overrun	Temp. °C.	% Overrun	Temp. °C.
140	−2.75	118	−3.20	115	−2.70	98	−2.85
132	−3.10	99	−3.55	100	−3.10	88	−3.20
119	−3.35	86	−4.00	90	−3.30	85	−3.30
92	−3.90	77	−4.30	81	−3.60	75	−3.60
83	−4.10	59	−4.80	68	−4.10	64	−3.90
62	−4.80			46	−4.65	48	−4.60
% Overrun = 38.8t + 245		% Overrun = 35.8t + 232		% Overrun = 29.4t + 189		% Overrun = 28.1t + 178	
Max. D. R. 143%		Max. O. R. 125%		Max. O. R. 115%		Max. O. R. 109%	

C. Mixes of 16% butter-fat, 8% milk-solids-not-fat and 14% sugar, 2500 lb. homogenization pressure.

% Overrun	Temp. °C.	% Overrun	Temp. °C.
115	−2.75	100	−2.60
109	−2.85	95	−2.70
96	−3.10	82	−2.90
85	−3.30	64	−3.60
69	−3.50	50	−3.90
60	−3.80		
% Overrun = 48.5t + 240		% Overrun = 40.0t + 202.5	
Max. O. R. 118%		Max. O. R. 107%	

D. Mixes of 12% butter-fat, 10% milk-solids-not-fat, 14% sugar and no gelatin.

1.—3500 lb. H. P.		2.—2500 lb. H. P.*		3.—1500 lb. H. P.	
% Overrun	Temp. °C.	% Overrun	Temp. °C.	% Overrun	Temp. °C.
111	−3.80	140	−.275	98	−3.90
98	−4.20	132	−3.10	88	−4.30
81	−4.80	119	−3.35	76	−4.40
74	−4.90	92	−3.90	60	−5.00
65	−5.10	83	−4.10		
		62	−4.80		
% Overrun = 36.4t + 252		% Overrun = 38.8t + 245		% Overrun = 36.4t + 243	
Max. O. R. 118%		Max. O. R. 143%		Max. O. R. 102%	

* Data from Series B No. 1.

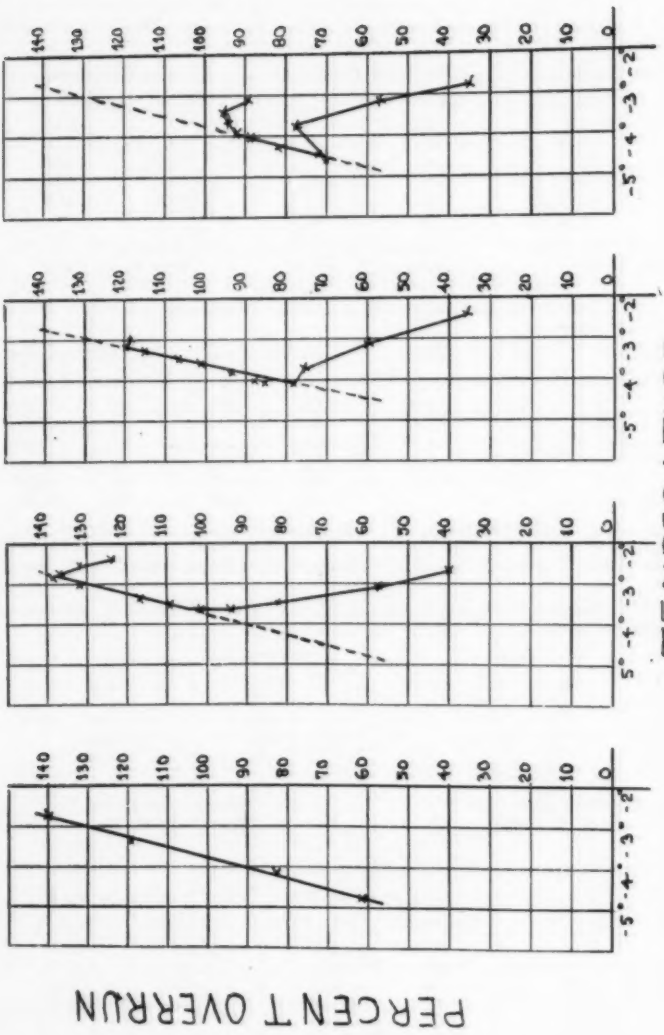

FIG. 2. FREEZING DATA FOR THREE PORTIONS OF A NORMAL ICE CREAM MIX IN RELATION TO THE OVERRUN-TEMPERATURE EQUILIBRIUM LINE.

the line is reached at a somewhat lower overrun and is not followed so far before the break occurs. The complete data are given in Table 2.

TABLE 2

Freezing data for three portions of a normal ice cream mix
(See Fig. 2)

B												
Time (Min.)	4	6*	8	10	12	14	16	18	20	22	24	26
Overrun	40	57	82	94	102	109	117	132	138	134	131	123
Temp. °C.	−2.7	−3.1	−3.5	−3.7	−3.6	−3.5	−3.35	−3.1	−2.9	−2.8	−2.6	−2.4
C												
Time (Min.)	4	6*	8*	10	12	14	16	18	20	22	24	26
Overrun	37	60	76	78	85	88	94	101	107	115	119	118
Temp. °C.	−2.4	−3.1	−3.75	−4.1	−4.2	−4.1	−3.9	−3.7	−3.55	−3.35	−3.1	−3.0
D												
Time (Min.)	4	6	8	10*	12	14	16	18	20	22	24	
Overrun	35	37	78	72	70	82	88	92	94	96	89	
Temp. °C.	−2.8	−3.2	−3.8	−4.5	−4.6	−4.25	−4.1	−3.9	−3.7	−3.4	−3.2	

* Indicates time brine was turned off.

In some of the other tests, when the freezings were carried to lower temperatures, the equilibrium line was reached before the brine was shut off. In such cases the line was followed down and back again as the temperature fell and rose. One low butter-fat mix with 0.5% gelatin, made from old condensed milk, was very unstable, as was the normal mix homogenized at 1500 pounds pressure. With these mixes it was impossible to stay on the equilibrium line for more than two minutes, that is, instability occurred when the mixes had been in the freezer for about fourteen minutes. Usually, however, departure from normal equilibrium did not occur until mixes had been in the freezer from 22 to 26 minutes. The stability of the different mixes may perhaps be expressed by indicating the number of minutes they are stable in the freezer under given freezing conditions but more work must be done upon this subject before final suggestions can be made.

That the instability is due to the action of the beaters seems to be shown conclusively by the fact that it was possible to freeze a mix to a low temperature, and, by passing comparatively warm brine through the freezer, to warm it up rapidly, and describe the complete equilibrium line before instability occurred.

The figures show conclusively that the straight line equilibrium curve between temperature and overrun is attained in this freezer during normal freezing if the mix is whipped long enough, and that the following of the curve for a period with rise in temperature is conditioned only by the stability of the mix to the continued action of the beaters.

To make sure that these relationships were not peculiar to this brine freezer, a considerable number of freezings were also carried out in a rapid freezing 20-quart direct expansion ammonia freezer with dasher speed of 200–R. P. M.

The same straight line temperature-overrun relationships were found with this freezer, but overruns were slightly higher, from 3 to 7%, and stability markedly less, so much so, that with a normally stable mix it was impossible to follow the straight line in whipping back, and a mix that was stable in the brine freezer for 22 to 26 minutes was stable in this freezer for only 12 to 14 minutes. It was found that stability could be imparted to the mix by freezing slowly, that is, by retarding the rate of evaporation of the ammonia. These phenomena are being investigated in detail and will be the subject of a future paper.

CONCLUSIONS

An equilibrium between temperature and the overrun obtainable in the freezing of ice cream mixes has been shown to exist. The equation of this relationship is that of a straight line. The equation must be qualified by stating the maximum overrun that can be attained in the mix, and probably further by a figure indicating the stability of the mix under the conditions of freezing. This figure would perhaps be a statement of the number of minutes that the mix could be beaten in the freezer before instability resulted. A departure from this equilibrium curve occurs also if mixes are frozen to too low a temperature.

By means of this information it is possible to express the whipping capacities of ice cream mixes and to evaluate differences brought about by the variation of factors incident to ice cream manufacture. The ice cream manufacturer may thus see how to control his freezings accurately and thus obtain the desired overrun in the shortest possible time. The information may also be applicable to the designing of freezers for the best performance.

American Dairy Science Association Announcements

B. W. HAMMER PANEGYRIC

It is gratifying to learn that former students of Doctor Hammer are honoring him at the completion of 25 years of distinguished service at Iowa State College. Professor C. B. Lane, chairman of the Hammer Commemoration Committee has written the following announcement letter to the editor and it will be of interest to all readers of the JOURNAL OF DAIRY SCIENCE.

"Doctor B. W. Hammer, Professor and Head of Dairy Bacteriology at the Iowa State College, has completed twenty-five years of distinguished service to dairy science and to the dairy industry in general. Subsequent to graduation from the University of Wisconsin Doctor Hammer continued his studies there under Doctor E. G. Hastings and was Assistant in Agricultural Bacteriology from 1908–1909. Then he was Bacteriologist, associated with Doctor M. P. Ravenel at the Wisconsin State Hygienic Laboratory at the University of Wisconsin, from 1909 to 1911, after which he joined the Dairy Industry Department at the Iowa State College in 1911. Since 1916 he has been in his present position as Head of Dairy Bacteriology at the Iowa Agricultural Experiment Station and Professor of Dairy Bacteriology. He managed to find time from a busy academic life to continue graduate study and research, and in 1920, he was awarded the degree Doctor of Philosophy from the University of Chicago.

"It is unnecessary to cite the significant contributions of Doctor Hammer in the field of Dairy Bacteriology and Chemistry; his revelations of important biological and chemical processes, significant in butter and other dairy products, are well known to those connected with dairy research or commercial dairying. Teaching, both graduate and under-graduate, must also be a pleasure to him, otherwise it is difficult to see how he can teach so effectively and excellently.

"To commemorate the occasion of Doctor Hammer's twenty-fifth anniversary his former students have written and published the 'B. W. Hammer Panegyric,' a volume primarily composed of original scientific treatises relating to dairy manufacture, bacteriology and chemistry. The volume contains 27 treatises by as many students and, in addition, several tributes by persons well known in the industry.

"The committee takes pleasure in announcing to you this significant anniversary and the publication of the Panegyric. Copies may be obtained at $2.50 each by ordering from the Collegiate Press, Inc., Iowa State College, or from C. B. Lane, Dairy Industry Department, Iowa State College."

Very truly yours,
C. B. Lane, Chairman
Hammer Commemoration Committee.

AMERICAN DAIRY SCIENCE ASSOCIATION

The Thirty-second Annual Meeting

Agricultural College Campus, University of Nebraska

Lincoln, Nebraska—June 21–25, 1937

GENERAL PROGRAM

Monday, June 21

1 P. M.–9 P. M.	General registration and room registration, Dairy Industry Building.

Tuesday, June 22

8 A. M.–9 P. M.	General registration and room registration, Dairy Industry Building.
12 NOON–1 P. M.	Lunch hour.
1:30 P. M.–4:30 P. M.	Ice Cream Judging Conference, Room 204, Dairy Industry Building. H. W. Gregory, Chairman, Purdue University. A. C. Dahlberg, Judge, New York Agricultural Experiment Station.
1:30 P. M.–4:30 P. M.	Reproduction Symposium, Dairy Barn. The Rôle of the Hormones in Reproduction with Special Reference to the Female Sex Hormones. T. S. Sutton, Ohio State University. The Preparation, Properties, and Use of Gonad-Stimulating Hormones. L. E. Casida, University of Wisconsin. Trichomoniasis Review. L. Van Es, University of Nebraska. Artificial Insemination—Demonstration. H. P. Davis and George W. Trimberger, University of Nebraska.
4:30 P. M.	Inspection of dairy barns and the dairy herd.
5:00 P. M.	Meeting of the Board of Directors, Room 207, Dairy Industry Building.
8:30 P. M.	Reception to members and guests by the University of Nebraska, Carrie Belle Raymond Hall, 540 North 16th Street.

Wednesday, June 23

8 A. M.–12 NOON	General registration and room registration, Dairy Industry Building.
8 A. M.–9:30 A. M.	Sectional Committee Meetings.

MANUFACTURING

Chemical Methods for the Analysis of Milk and Dairy Products, Room 301, Dairy Industry Building.

L. C. Thomsen (Chairman).

Dairy Products Quality, Room 207, Dairy Industry Building.
W. H. E. Reid (Chairman).

Bacteriological Methods for the Analysis of Milk and Dairy Products, Room, 303, Dairy Industry Building.
H. Macy (Chairman).

Judging Dairy Products, Room 204, Dairy Industry Building.
H. W. Gregory (Chairman).

Revision of Score Cards for the Sanitary Inspection of Dairy Farms and Milk Plants, Room 206, Dairy Industry Building.
C. J. Babcock (Chairman).

Methods of Determining the Curd Tension of Milk, Room 101, Animal Husbandry Hall.
L. H. Burgwald (Chairman).

Feasibility of Establishing and Maintaining a Loose-leaf Manual of Laboratory Methods, Room 303, Dairy Industry Building.
H. Macy (Chairman).

PRODUCTION

Breeds Relations, Room 205, Animal Husbandry Hall.
S. M. Salisbury (Chairman).

Student Judging Contest, Room 103, Animal Husbandry Hall.
I. W. Rupel (Chairman).

Measuring Results of Pasture Investigations, Room 208, Animal Husbandry Hall.
R. H. Lush (Chairman).

Methods of Experimentation and Analysis, Room 208-A, Dairy Industry Building.
A. E. Perkins (Chairman).

8 A. M.–9:30 A. M. Inspection of Extension Exhibits, Room 102, Animal Husbandry Hall.

9:00 A. M.–12 NOON Special Recreation for Children, College Activities Building.

9:30 A. M.–12 NOON General Session, College Activities Building.
R. R. Graves, presiding.
Address of Welcome.
E. A. Burnett, Chancellor, University of Nebraska.
Presidential Address.
R. R. Graves, Bureau of Dairy Industry, U. S. D. A.
The Eleventh World's Dairy Congress.
J. C. Marquardt, New York Agricultural Experiment Station.

A Teacher Interprets Research.
W. H. Morton, University of Nebraska.
Breeding and Feeding Dairy Cattle in Denmark.
H. Wenzel Eskedal, Landøkonomisk Forsøgslaboratorium, Copenhagen, Denmark.

12 NOON–1 P. M. Lunch.

1:30 P. M.–4 P. M. A tour for ladies of the Fine Arts Department and the University Art Gallery has been arranged to be conducted by a member of the Fine Arts staff. This will be followed by a tour of the University Natural History Museum including the Hall of the Elephants conducted by a member of the museum staff. Meet at Morrill Hall, 14th and U Sts.

1:30 P. M.–4:15 P. M. Special recreation for children, College Activities Building.

1 P. M.–4:15 P. M. Production Section, Room 303, Dairy Industry Building.
Dairy Management and Physiology.
Manufacturing Section, Room 204, Dairy Industry Building.
Bacteriology.
Manufacturing Section, Room 301, Dairy Industry Building.
Ice Cream and Milk By-products.
Extension Section, Room 208, Animal Husbandry Hall.
Sire and Feed Committees.

4:15 P. M. Visit to University Natural History Museum—Elephant Hall, Morrill Hall, City Campus.

8:30 P. M. Entertainment, College Activities Building.

Thursday, June 24

8:00 P. M.–12 NOON Production Section, Room 303, Dairy Industry Building.
Feeding.
Manufacturing Section, Room 301, Dairy Industry Building.
Butter.
Extension Section, Room 208, Animal Husbandry Hall.
Exhibits and Testing Committee.

9:00 A. M.–12 NOON Special recreation for children, College Activities Building.

12 NOON–1 P. M. Lunch for men, compliments of Dairy Husbandry Department, College Activities Building.

1:30 P. M. Ladies tour of Nebraska State Capitol and tea.

1:30 P. M.–4:15 P. M. Special recreation for children, College Activities Building.

1 P. M.–4:15 P. M.	Production Section, Room 303, Dairy Industry Building. Vitamins. Manufacturing Section, Room 301, Dairy Industry Building. Cheese. Extension Section, Room 208, Animal Husbandry Hall. Four H Club and Quality Committees. Instruction Section, Room 204, Dairy Industry Building.
4:15 P. M.	Visit to Nebraska State Capitol.
4:15 P. M.	Committee meetings.
6:30 P. M.	Annual Banquet followed by cards and dancing at Lincoln Hotel.

Friday, June 25

8 A. M.–10 A. M.	Production Section, Room 303, Dairy Industry Building. Business Meeting. Genetics. Manufacturing Section, Room 301, Dairy Industry Building. Business Meeting. Chemistry and Economics. Extension Section, Room 208, Animal Husbandry Hall. Business Meeting. American Dairy Cattle Club.
10 A. M.–12 NOON	General Session, College Activities Building. Business Session. Borden Awards. Resumé of awards by chairman of committees. H. B. Ellenberger—for production. H. A. Ruehe—for manufacturing. Presentation of awards and medals by representative of Borden Company. Speeches on work by recipients of awards.

SECTION PROGRAMS

EXTENSION SECTION

Wednesday morning, June 23

C. W. BLACKMAN, *Chairman*

Program Committee Extension Section

Earl Shultz, Iowa State College, E. J. Perry, New Jersey College of Agriculture, and M. L. Flack, Chairman, University of Nebraska.

8 A. M.–9:30 A. M.—INSPECTION OF EXHIBITS

Room 102, Animal Husbandry Hall

J. W. LINN, *Chairman*

Wednesday afternoon, June 23

1 P. M.–3 P. M.—SIRE COMMITTEE REPORT

Room 208, Animal Husbandry Hall

J. F. KENDRICK, *Chairman*

E1—Conducting organized dairy cattle breeding programs through bull associations. R. G. Connelly, Virginia Polytechnic Institute.
Discussions by 1. C. S. Rhodes, University of Illinois.
2. S. J. Brownell, Cornell University.
3. Ivan H. Loughary, University of Idaho.
4. E. A. Gauntt, New Jersey College of Agriculture.

E2—Dairy sire exchange lists. Warren Gifford, University of Missouri.
Discussions by 1. J. G. Hays, Michigan State College.
2. G. W. Vergeront, University of Wisconsin.
3. O. J. Hill, State College of Washington.
4. Floyd Arnold, Iowa State College.

E3—Using D. H. I. A. records in conducting dairy cattle breeding programs. E. E. Heizer, Ohio State University.
Discussions by 1. S. J. Brownell, Cornell University.
2. Gordon Dickerson, University of Wisconsin.
3. Jay L. Lush, Iowa State College.

E4—Forum: D. H. I. A. identification and permanent record project. J. H. Kendrick, Bureau of Dairy Industry, U. S. D. A.

3:00 P. M.–4:15 P. M.—FEEDING COMMITTEE REPORT

Room 208, Animal Husbandry Hall

R. E. HORWOOD, *Chairman*

E5—A feed insurance program with trench silos. V. L. Gregg, University of Arkansas.

E6—An extension program in dairy cattle feeding and feed crop production. K. L. Turk and W. T. Crandall, Cornell University.

E7—Establishing and conducting a dairy pasture improvement program in Missouri. M. J. Regan, University of Missouri.

E8—A new method for conducting a feed meeting. J. G. Hays, Michigan State College.

EXTENSION SECTION

Thursday morning, June 24

8 A. M.–9:30 A.M.—EXTENSION EXHIBITS

Room 102, Animal Husbandry Hall

J. W. LINN, *Chairman*, Kansas State College
J. G. HAYS, Michigan State College

R. A. CAVE, South Dakota State College
DON CORBETT, University of Maine.
M. L. FLACK, University of Nebraska

Exhibits from several states showing dairy extension methods in teaching will be on display with explanatory labels during the entire meeting. The above committee and specialists will be present during this period to discuss and explain the exhibits from their own states.

9:30 A.M.–12 NOON—TESTING COMMITTEE REPORT

Room 208, Animal Husbandry Hall

FLOYD JOHNSON, *Chairman*

E9—Subsidizing testing from the state office. J. W. Linn, Kansas State College.
E10—Subject matter included in testers' training courses. Floyd J. Arnold, Iowa State College.
E11—Getting permanent records filled out for dairy herd improvement association members. C. R. Gearhart, Pennsylvania State College.
E12—What can be eliminated from the tester's work to make possible newer developments. A. B. Nystrom, Bureau of Dairy Industry, U. S. D. A.
E13—Uniform rules governing the operation of dairy herd improvement associations. Floyd Johnston, Iowa State College.
E14—Dairy herd improvement association publicity. E. C. Scheidenhelm, Michigan State College.

EXTENSION SECTION

Thursday afternoon, June 24, 1 P. M.–4 P. M.

Four H Club Committee Report, Room 208, Animal Husbandry Hall

G. M. HARRIS, *Chairman*

E15—Methods of financing 4-H dairy calf club members. J. W. Linn, Kansas State College.
E16—Applying the Danish system of judging to dairy 4-H club work. D. M. Seath, Kansas State College, and G. M. Harris, University of Kentucky.
E17—4-H club demonstrations for improvement of quality. E. A. Gauntt, New Jersey College of Agriculture.

Quality Committee Report

C. J. BABCOCK, *Chairman*

E18—A quality improvement project for milk and cream. C. J. Babcock, Bureau of Dairy Industry, U. S. D. A.

Discussion by C. A. Smith, Colorado State College.

E19—Outline of Colorado quality improvement project. C. A. Smith, Colorado State College.

EXTENSION SECTION

Friday morning, June 25, 8 A. M.–10 A. M.

Room 208, Animal Husbandry Hall

C. L. BLACKMAN, *Chairman*

Business session

E20—American Dairy Cattle Club, J. Rockerfeller Prentice, President of American Dairy Cattle Club.

INSTRUCTION SECTION

Thursday afternoon, June 24, 1 P. M.–4: 15 P. M.

Room 204, Dairy Industry Building

C. Y. CANNON, *Presiding*

(Papers limited to 12 minutes)

I1—Trends in dairy instruction. C. E. Wylie, University of Tennessee.

I2—Junior colleges and their influence on dairy education. C. L. Roadhouse, University of California.

I3—Factors in the retention of knowledge. E. N. Hansen, Iowa State College.

I4—Rapid calculation of rations by means of a pony. P. T. Dix Arnold, University of Florida.

I5—College creameries. Thomas B. Harrison and C. E. Wylie, University of Tennessee.

I6—A course in milk and public health. H. O. Henderson, University of West Virginia.

5-MINUTE REST PERIOD

I7—The desirability of an advanced course in dairy industry as a requirement for agricultural students. Kenneth M. Renner, Texas Technological College.

I8—Extramural courses in dairy husbandry. H. A. Ruehe, University of Illinois.

I9—Undergraduate dairy seminar. A. A. Borland, Pennsylvania State College.

I10—Practical dairy industry experience and the scholastic record. E. F. Goss, Iowa State College.

I11—The placement of dairy graduates. M. Mortensen, Iowa State College.

MANUFACTURING SECTION

Wednesday afternoon, June 23, 1 P. M.–4:15 P. M.

Room 204, Dairy Industry Building

H. MACY, *Presiding*

BACTERIOLOGY

(Papers limited to 12 minutes)

M1—The isolation of the citric acid fermenting streptococci from butter cultures. H. C. Olson, Iowa State College.

M2—The correlation between the organisms found microscopically in butter serum and the grade of cream from which the butter was made. Theodore Hedrick, Montana State College.

M3—The detection of mastitis by the brom-thymol-blue test, leucocyte count, and the microscopic examination of milk. A. C. Fay, H. W. Cave and F. W. Atkeson, Kansas State College.

M4—A combined pasteurizer, cooler and incubator for mother starter. G. H. Wilster and F. E. Price, Oregon State Agricultural College.

M5—Studies upon a bacteriophage inhibitory to *Streptococcus lactis*. F. E. Nelson, University of Minnesota, and B. W. Hammer, Iowa State College.

M6—The dye concentration in culture media employed for the analyses of Escherichia-aerobacter members in milk. H. D. McAuliffe and A. A. Borland, Pennsylvania State College.

5-MINUTE REST PERIOD

M7—The effect of salts on the growth of bacteria in milk. C. S. Mudge and T. G. Anderson, University of California.

M8—Comparative studies on bacterial milk counts in various types of media incubated at 20°, 30°, and 37° C. J. Drexel Dennis and Harry H. Weiser, Ohio State University.

M9—A study of comparative methods and media used in the microbiological examination of creamery butter—I. Yeast and mold counts. G. W. Shadwick, Jr., Beatrice Creamery Co.

M10—Proposed standard for the yeast and mold count of salted butter made from sour cream. E. H. Parfitt, Purdue University.

M11—Studies on *Oospora lactis*. H. Macy and D. L. Gibson, University of Minnesota.

M12—*Pseudomonas fragi* and its importance in dairy products. H. F. Long and B. W. Hammer, Iowa State College.

MANUFACTURING SECTION

Wednesday afternoon, June 23, 1 P. M.–4:15 P. M.

Room 301, Dairy Industry Building

P. H. TRACY, *Chairman*

ICE CREAM AND MILK BY-PRODUCTS

(Papers limited to 12 minutes)

M13—The manufacture of sweetened condensed whey and its use in foods. G. A. Ramsdell and B. H. Webb, Bureau of Dairy Industry, U. S. D. A.

M14—The manufacture of non-foaming casein. G. A. Richardson, N. P. Tarassuk and L. B. Fry, University of California.

M15—Flexible milk plants. W. E. Guest and R. W. Balderston, W. E. Guest and Co.

M16—Sonic homogenization of milk and of ice cream mixes. Leslie A. Chambers, Eldridge Reeves Johnson Foundation, University of Pennsylvania.

M17—A suggested method of evaluating homogenization efficiency by improved photomicrography. A. W. Farrall and R. L. Hanson, Creamery Package Manufacturing Co.

M18—A simplified solids tester for ice cream. Kenneth M. Renner, Texas Technological College.

5-MINUTE REST PERIOD

M19—Effect of certain salts on properties of ice cream mixes. J. I. Keith, C. W. Rink and Earl Weaver, Oklahoma A. & M. College.

M20—Power requirements for freezing ice cream. W. J. Caulfield, C. K. Otis and W. H. Martin, Kansas State College.

M21—Sogo ice cream. Thos. B. Harrison and C. E. Wylie, University of Tennessee.

M22—Flavor defects encountered in strawberry ice cream prepared with commercial dry skim milk and condensed milk from stainless steel pans. E. W. Bird, J. J. Willingham and C. A. Iverson, Iowa State College.

M23—Some factors affecting the serving and dipping qualities of ice creams. W. H. E. Reid and W. S. Arbuckle, University of Missouri.

MANUFACTURING SECTION

Thursday morning, June 24, 8 A. M.–12 NOON

Room 301, Dairy Industry Building

P. H. TRACY, *Chairman*

BUTTER

(Papers limited to 12 minutes)

M24—Volumetric method for determination of diacetyl. H. A. Ruehe and W. J. Corbett, University of Illinois.

M25—The influence of heated testers and composite tests on the Babcock test. P. S. Lucas, Michigan State College.

M26—Removal of French weed flavor from cream. W. B. Combs and S. T. Coulter, University of Minnesota.

M27—Effect of temperature on the rate of deterioration of cream. W. H. Martin, A. C. Fay and W. J. Caulfield, Kansas State College.

M28—Some aspects of the reduction of acidity in cream for the manufacture of butter. E. W. Bird, N. E. Fabricius and D. F. Breazeale, Iowa State College.

M29—Studies on the keeping quality of butter made from sour cream. J. C. Flake and E. H. Parfitt, Purdue University.

5-MINUTE REST PERIOD

M30—Notes on problems confronting the industry on quality improvement of creamery butter. M. E. Parker, Beatrice Creamery Co.

M31—Overcoming the gummy body of butter caused by feeding cottonseed meal. J. I. Keith, C. W. Rink and A. H. Kuhlman, Oklahoma A. & M. College.

M32—The lactic acid content in butter. B. E. Horrall and W. F. Epple, Purdue University.

M33—The pH range of centralizer butter. W. H. Brown and E. H. Parfitt, Purdue University.

MANUFACTURING SECTION

Thursday afternoon, June 24, 1 P. M.–4:15 P.M.

Room 301, Dairy Industry Building

P. H. TRACY, *Chairman*

CHEESE

(Papers limited to 12 minutes)

M34—Microflora of cheese slime. H. Macy and J. A. Erekson, University of Minnesota.

M35—Making cheddar cheeses from low curd tension milk. J. C. Marquardt and G. J. Hucker, New York Agricultural Experiment Station.

M36—Curd tension measurements. L. H. Burgwald and T. V. Armstrong, Ohio State University.

M37—The effect of varying storage temperatures and the effect of coverings on the ripening of cheddar cheese. W. G. McCubbin and E. L. Reichart, University of Nebraska.

M38—Cheese freezing and curing investigations. J. C. Marquardt, New York Agricultural Experiment Station.

M39—Studies relative to an open flame method for determining the moisture content of cheddar cheese. I. A. Gould, Michigan State College.

M40—Homogenization of milk for blue cheese. C. B. Lane and B. W. Hammer, Iowa State College.

5-MINUTE REST PERIOD

M41—Studies on the ripening of blue or American Roquefort cheese. S. T. Coulter, W. B. Combs and Spencer George, University of Minnesota.

M42—Studies on the ripening of blue cheese. C. B. Lane and B. W. Hammer, Iowa State College.

M43—The influence of steapsin on the rate of ripening blue or American Roquefort cheese. W. B. Combs and S. T. Coulter, University of Minnesota.

M44—A photomicrographic study of processed cheese. Hugh L. Templeton, University of Wisconsin.

M45—Influence of manufacturing methods upon the acidity of brick cheese. D. W. Spicer and Walter V. Price, University of Wisconsin.

M46—Relation between acid defects and hydrogen ion concentration in brick cheese. W. V. Price and D. W. Spicer, University of Wisconsin.

MANUFACTURING SECTION

Friday morning, June 25, 8 A. M.–10 A. M.

Room 301, Dairy Industry Building

P. H. TRACY, *Chairman*

Business Meeting

CHEMISTRY AND ECONOMICS

(Papers limited to 12 minutes)

M47—Bound water and its relation to dairy products. Harry Pyenson and C. D. Dahle, Pennsylvania State College.

M48—The phosphatase test for the efficiency of pasteurization. A. B. Storrs and L. H. Burgwald, Ohio State University.

M49—The significance of ammonia in milk: a practical method for its determination. A. E. Perkins, Ohio Agricultural Experiment Station.

M50—The application of Ritter's test for the detection of copper in milk and dairy products. Jules Turgeon, V. C. Stebnitz and H. H. Sommer, University of Wisconsin.

M51—Production regulations necessary to supply the milk market. B. B. Derrick, Maryland and Virginia Milk Producers Association.

PRODUCTION SECTION

Wednesday afternoon, June 23, 1 P. M.–4:15 P. M.

Room 303, Dairy Industry Building

F. W. ATKESON, *Chairman*

DAIRY MANAGEMENT

(Papers limited to 12 minutes)

P1—A study of the methods of sampling milk for butterfat tests where a combine milker is used. K. S. Morrow and H. C. Moore, University of New Hampshire.

P2—Changes occurring in the freshening dates from year to year of cows in Iowa testing associations. C. Y. Cannon and D. L. Espe, Iowa State College.

P3—A new visible system of dairy herd books. F. W. Atkeson and H. W. Cave, Kansas State College.

P4—Estimating live weight from chest girth of dairy cattle of unknown age. S. Brody and A. C. Ragsdale, University of Missouri. H. P. Davis, University of Nebraska.

PHYSIOLOGY

P5—Is the calcium: phosphorus ratio of common mineral mixtures suited to dairy cattle? G. Bohstedt, University of Wisconsin.

P6—Essentiality of cobalt in bovine nutrition. W. M. Neal and C. F. Ahmann, University of Florida.

5-MINUTE REST PERIOD

P7—Fermentation energy losses in dairy cattle. L. E. Washburn, University of Missouri.

P8—Acetonemia and ketonuria in dairy cows under farm conditions. C. W. Duncan, C. F. Huffman and H. A. Tobin, Michigan State College.

P9—Certain points in the physiological processes of the cow. R. B. Becker, University of Florida.

P10—The adrenal cortical hormone in relation to lactation. E. T. Gomez and C. W. Turner, University of Missouri.

P11—The rôle of the central nervous system in the hormonal control of lactation. R. P. Reece and C. W. Turner, University of Missouri.

P12—The effect of thyroxine on milk and fat production. H. A. Herman, W. R. Graham and C. W. Turner, University of Missouri.

P13—On the carbohydrate and nitrogen metabolism of the mammary gland. W. R. Graham, University of Missouri.

PRODUCTION SECTION

Thursday morning, June 24, 8 A. M.–12 NOON

Room 303, Dairy Industry Building

F. W. ATKESON, *Chairman*

FEEDING

(Papers limited to 12 minutes)

P14—The value of corn sugar in the grain mixture of dairy calves. Clifton A. Ward, C. Y. Cannon and D. L. Espe, Iowa State College.

P15—Replacing whole milk in the calf ration. R. T. Parkhurst, National Oil Products Co.

P16—Hay consumption of Holstein calves. H. S. Willard, University of Wyoming.

P17—Development of calves on prairie hay when fed milk from dams on similar rations. H. W. Cave, W. H. Riddell and J. S. Hughes, Kansas State College.

P18—Limited prairie hay rations and avitaminosis in dairy heifers. A. H. Kuhlman, Andrew Nalbandov and Earl Weaver, Oklahoma A. & M. College.

P19—Limited grain feeding of dairy cattle. C. E. Wylie and L. R. Neel, University of Tennessee.

P20—Some experiences in feeding cattle on rations devoid of roughage. T. W. Gullickson, University of Minnesota.

5-MINUTE REST PERIOD

P21—A comparison of pasture returns from actual grazing and clip plot methods. I. R. Jones, H. P. Ewalt and J. R. Haag, Oregon State Agricultural College.

P22—The losses of dry matter in corn silage stored in snow-fence silos and the cost per ton of storage. J. B. Shepherd, Bureau of Dairy Industry, U. S. D. A.

P23—The apparent digestibility and feeding value of apple-alfalfa silage. J. C. Knott and R. E. Hodgson, State College of Washington.

P24—Mungbean silage for milk production. A. H. Kuhlman, Andrew Nalbandov and Earl Weaver, Oklahoma A. & M. College.

P25—Molasses grass silage as the sole roughage diet for milk production and growth of dairy animals. C. B. Bender, J. W. Bartlett, H. H. Tucker and J. Mixner, New Jersey Agricultural Experiment Station.

P26—Relation of grass silage to the color, vitamin C and flavor in milk from individual cows. O. F. Garrett, C. B. Bender and H. H. Tucker, New Jersey Agricultural Experiment Station.

PRODUCTION SECTION

Thursday afternoon, June 24, 1 P. M.–4:15 P. M.

Room 303, Dairy Industry Building

F. W. ATKESON, *Chairman*

VITAMINS

(Papers limited to 12 minutes)

P27—Technique used in studying the vitamin A requirements of dairy cattle. L. A. Moore, Michigan State College.

P28—The carotene and color content of home-grown roughage feeds and the relation of these rations to the carotene, color and vitamin A activity of the butterfat. R. E. Hodgson, J. C. Knott, H. K. Murer and R. R. Graves, Bureau of Dairy Industry, U. S. P. A. and State College of Washington.

P29—Effect of carotene intake on the carotene and vitamin A content of butter. H. J. Smith and E. B. Powell, Ralston Purina Co.

P30—An attempt to remove the vitamin A suppressing factor in soybean oil by adsorbents. S. M. Hauge, J. W. Wilbur and J. H. Hilton, Purdue University.

P31—The relation of A. I. V. alfalfa silage and the carotene content of milk. W. E. Petersen, J. B. Fitch and N. N. Allen, University of Minnesota.

P32—Effect of molasses and A. I. V. silages on the carotene and vitamin A content and growth-promoting quality of milk. D. M. Hegsted and G. Bohstedt in collaboration with C. A. Elvehjem, E. B. Hart, W. H. Peterson and I. W. Rupel, University of Wisconsin.

5-MINUTE REST PERIOD

P33—Oxidized milk flavor as related to carotene, lecithin and vitamin C. C. H. Whitnah, W. H. Martin and G. H. Beck, Kansas State College.

P34—The vitamin D requirement (U. S. P. Units) for growth and well-being of calves from birth to six months of age. S. I. Bechdel, Neal W. Hilston and N. B. Guerrant, Pennsylvania State College.

P35—Studies in methods of concentrating the vitamin D of butterfat for assay purposes. G. C. Wallis, South Dakota State College.

P36—X-ray technique for studying rickets in calves. S. I. Bechdel, Neal W. Hilston, Pennsylvania State College, and Robert F. Light, Fleischmann Laboratories.

P37—Effects of vitamin D deficiency on mature dairy cows. G. C. Wallis, South Dakota State College.

P38—Irradiation of milk; the interrelation of radiation intensity and milk film capacity. H. H. Beck, H. C. Jackson and K. G. Weckel, University of Wisconsin.

PRODUCTION SECTION

Friday morning, June 25, 8 A. M.–10 A. M.

Room 303, Dairy Industry Building

F. W. ATKESON, *Chairman*

Business meeting

GENETICS

(Papers limited to 12 minutes)

P39—Hair pigment. W. E. Petersen and W. M. Sandstrom, University of Minnesota.

P40—The relation of the endocrine glands to the inheritance of milk secretion. C. W. Turner, University of Missouri.

P41—Evaluation of different measures of inherited producing ability in dairy cattle. Gordon E. Dickerson, University of Wisconsin.

P42—Are culls really culls? D. M. Seath, Kansas State College.

P43—Differences between records, real productivity and breeding values of dairy cows. Jay L. Lush and Floyd Arnold, Iowa State College.

VOLUME XX JUNE, 1937 NUMBER 6

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Certified Milk

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SPECIAL PUBLICATIONS

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United States Department of Agriculture

ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

BACTERIOLOGY

156. Van Oijen Modification of the Frost Little Plate Method. A Critical Investigation of Van Oijen's Test for the Bacterial Content of Milk Samples. H. BARKWORTH, South-Eastern Agr. College, Wye, Kent. J. Dairy Research *8*, p. 244, Sept. 1936.

The Van Oijen test makes possible an estimate of small numbers of organisms in milk and is used in Holland for the official control of "certified" milk.

The colony counting error of the Van Oijen test was found to be about the same as that of the plate count.

From the results of quintuplicate tests it is recommended that 24 hours incubation at 28° C. be used for the Van Oijen test and 72 hours at 37° C. for the plate test.

Thirty-one low count samples (3000–30,000 per ml.) and thirty-one high count samples (30,000–300,000 per ml.) were tested in quintuplicate by both methods, and statistical examination of the results shows that at both levels of count the Van Oijen test is significantly more accurate.

In the Van Oijen test the results are based on a larger amount of milk than the plate test and it is held that in comparing various methods of assessing the numbers of organisms in milk the size of sample may have a great effect upon accuracy. H.A.B.

157. A Pigment-Producing Organism (Pseudomonas Sp.) Isolated from Discolored Butter. E. R. HISCOX, National Inst. for Research in Dairying, Univ. of Reading. J. Dairy Research *8*, p. 238, Sept., 1936.

An organism causing a dark discoloration in butter containing 0.38–0.55 per cent salt was studied. At 15° C. a brownish color developed and diffused through the medium. At lower temperatures this was mixed with a blue-black pigment, insoluble in water, brine, chloroform, ether, ethyl and methyl alcohol, butterfat, and dilute sulphuric and acetic acids, but soluble in formalin. During growth of the organism the blue-black pigment was excreted from the cells and deposited as irregular aggregates of platelets varying considerably in size and shape. The nature of the source of the nitrogen influenced the development of this pigment, proteose appearing to be inhibitory. The brown pigment produced at higher temperatures may probably be attributed to the action of ammonia produced by the organism.

The organism, a rod with rounded ends, non sporing, actively motile and gram negative was regarded as a species of Pseudomonas. In litmus milk it

produced alkalinity with bleaching and digestion at all temperatures up to 30° C. At 1–3° C. the action was very slow, a dark ring developing to a depth of about ½ inch. No growth was obtained on standard agar, yeast-dextrose agar, beerwort agar, bean agar and potato. The first two media became satisfactory for growth with the addition of ½ per cent NaCl. With 5 per cent NaCl growth became very scanty. H.A.B.

158. Biennial Review of the Progress of Dairy Science. Section E. The Disease of Dairy Cattle. S. J. EDWARDS, The Hannah Dairy Research Inst., Kirkhill, Ayr. J. Dairy Research *8*, p. 291, Sept., 1936.

The literature regarding chronic streptococcus mastitis, contagious abortion and tuberculosis is reviewed—64 references are cited on mastitis, 90 on contagious abortion and 52 on tuberculosis. H.A.B.

BUTTER

159. Changes in Acetylmethylcarbinol Plus Diacetyl Content of Butter. WALTER L. SLATTER, Graduate Student, Iowa State College, Ames, Iowa. Nat. Butter and Cheese J. *27*, 20, p. 20, Oct. 25, 1936; 21, p. 18, Nov. 10, 1936.

The aroma of butter is due to the presence of diacetyl, the source of which is acetylmethylcarbinol. The sum of both substances in butter is an indication of the extent of desirable flavor development. The acetylmethylcarbinol plus diacetyl ($Amc + Ac_2$) was studied to determine the amounts produced and the factors influencing its production in salted and unsalted butter. All but one lot of butter was made from neutralized and pasteurized sweet cream to which was added butter culture. The culture contained added citric acid. Measurements of $Amc + Ac_2$ were made at intervals after churning. The following conditions favored or accompanied the production of $Amc + Ac_2$: unsalted butter; high temperatures of storage; decreasing pH; the use of 10 to 20 per cent culture; and the addition of citric acid fermenting streptococci to the cream. A decrease in $Amc + Ac_2$ occurred after the maximum was attained although the presence of salt tended to retard this decrease. Variations in production of $Amc + Ac_2$ were attributed to differences in cultures. W.V.P.

160. How American Creamerymen Advertise Butter. (Anonymous.) Nat. Butter and Cheese J. *27*, p. 6, Nov. 25, 1936.

A survey of butter manufacturers shows that 88.88 per cent of those responding use the newspaper and store signs as advertising media. About one-third of the advertising dollar is spent in newspapers, and one-quarter in store signs. Other media include the radio, house organs, outdoor advertising, street car signs, calendars, pencils, and other means. Relative impor-

tance of types of advertising and expenditure on each type are shown in tables. Advertising must be backed by quality of product. W.V.P.

161. Studies in Surface Flavour in Butter. W. H. SPROULE AND F. W. HAMILTON, Dept. of Dairying, Ont. Agr. College, Guelph.) Can. Dairy and Ice Cream J. *16*, p. 19, Feb., 1937.

This problem was studied from the standpoint of contamination of the butter by wash waters.

In studies of the chlorination of wash water it was suggested that the concentration of chlorine may properly vary from 5 to 10 p.p.m. depending upon the organic content of the water.

The filter used in the test was an efficient means of removing bacteria from water provided it was properly operated.

Nevertheless, there was no correlation between filtration of wash water available in these experiments and the keeping quality of butter.

J.C.H.

162. Starter vs. Non-Starter Butter. C. H. PARSONS AND F. W. BOUSKA. Am. Creamery and Poultry Prod. Rev. *81*, p. 364, Jan. 8, 1936.

Manufacturers of butter, in many cases, have doubted whether the cost of starter is justified by the results it produces in butter. Results secured in one hundred trials showed that the addition of starter to neutralized cream increased the score from 0.2 to 0.5 of one point and that the keeping quality of butter made with starter was somewhat better. It was possible to make a more uniform butter by the use of starter and to produce a more desirable flavor. Starter improved the butter from a 90–91 score cream to a greater extent than from 89 score cream. Whether this beneficial effect is great enough to warrant use of starter is a problem, the answer to which must be decided by local conditions.

Supplementing Mr. Parson's observations, Mr. Bouska advised that his observations convinced him of the improvement of butter through the use of starter. This improvement in keeping quality he believes to be due to the presence of lactic acid and suggests for satisfactory butter a pH range of 5.8 to 6.8. To reduce the cost of starter two alternatives are mentioned: (1), using half the usual amount of starter plus 0.15 pounds citric acid per 100 pounds starter; and, (2), working starter into the butter at working, and at the rate of no more than two to eight pounds per 1000 pounds of butter. P.S.L.

163. Analysis of Data fom National Butter Scoring Contest of 1934. S. L. TUCKEY AND P. H. TRACY. Am. Creamery and Poultry Prod. Rev. *81*, p. 844, April 8, 1936.

This article gives a detailed analysis of results secured in the 1934 annual butter scoring of the American Creamery Buttermaker's Association. Prac-

tically all fresh entries, 94.7 per cent, were made from sweet cream. In the storage class 93.5 per cent of the entries were made from sweet cream. With fresh butter samples, 83 per cent were made with cream testing 0.18 of 1 per cent, or under, acidity, and with storage butter 78 per cent. Several samples were neutralized but in most cases the initial acidity of the cream was so low as to make comparisons with the usual conditions impossible. No difference in keeping quality could be noted from the use of starter. Quality appeared to be due chiefly to the quality of the raw cream used. P.S.L.

Other abstracts of interest are numbers 157, 168, 171, 179, 181, 184, 185, 188, and 189.

CHEESE

164. Consumer Preferences for Cheese. Asher Hobson and Marvin A. Schaars, University of Wisconsin, Madison. Wis. Agr. Exp. Sta. Research Bul. 128, Oct., 1935.

University dining halls offered 650 students several kinds and qualities of cheese. Preferences were determined by per capita consumption. First choice of men was mild American, of women processed American. Other choices indicated lack of discrimination when compared with trade standards of quality. Among 6770 adult customers in retail stores in six cities, 48 per cent chose mild rather aged American samples which were offered to them to taste; 61 per cent chose processed American over aged American, women decidedly preferring the processed American; $\frac{2}{3}$ chose processed to natural Brick; and 55 per cent men and 63 per cent women selected processed over natural Swiss. Farm residents showed a greater preference for processed cheese. Mild, aged and processed American cheese was served to selected groups in non-university dining rooms day after day to see if original tastes would change. If processed was not chosen early in the 12 to 15 weeks' period of the test it eventually was consumed in the largest amounts. Some menus stimulated consumption of cheese. Interview with hotel managers and consumers showed personal reaction toward cheese as a food; the economy of cheese; ways of using it; and buying practices. Neither the retailers nor consumers know the quality of cheese. The bulletin contains 31 tables and 10 figures. W.V.P.

165. Packaging American Cheese. Walter V. Price, Univ. of Wisconsin, Madison. Wis. Agr. Exp. Sta. Research Bul. 130, Nov., 1935.

Fast-freezing of cured cheese in small packages seems to be a practical method of modernizing distribution of natural cheese. Cheese is selected and packaged at the most desirable stage of ripening. Quality and uniformity can, therefore, be identified by brand name. The package recommended is attractive, sanitary, and convenient for the consumer. Methods

of packaging, storing, defrosting, and merchandising are discussed in detail. The bulletin contains 9 tables, 11 figures and 39 illustrations.

W.V.P.

166. Observations on the Ripening of Cheeses Made from Raw and Pasteurized Milk. I. R. SHERWOOD, Dairy Research Inst. (N. Z.), Palmerston North, N. Z. J. Dairy Research *8*, p. 271, Sept., 1936.

The investigation was designed to show whether chemical changes produced in milk by pasteurization at various temperatures are sufficient to modify the proteolytic action of rennin, pepsin and trypsin on milk and on the cheese curd when bacterial action was inhibited by the addition of 2 per cent of chloroform.

To raw skimmilk and to skimmilk from the same bulk sample pasteurized for 30 minutes at temperatures of 100° F. to 212° F. were added 1.4 per cent rennet (factor sample), 0.6 per cent pepsin, 0.3 per cent and 0.1 per cent trypsin. The action of rennet and pepsin on the milk during 5 days at 20° C. was slightly reduced by heating the milk up to 140–160° F. while higher temperatures again increased proteolysis. In the case of the trypsin proteolysis during one day at 20° C. was markedly increased by increased heating temperatures up to 170° F. The change was especially marked over the temperature range of 150–160° F. By experiments, in which only the whey portion of the milk was heated and the milk then reconstituted, it was found that the change was due, not to an alteration of the casein, but to some change in the whey portion of the milk.

When cheese was made from raw milk and the same milk pasteurized at 165° F. and 175° F. it was found that the protein breakdown in the cheese proceeded more rapidly in the raw milk cheese than in the pasteurized milk cheese. The same was true when chloroform was added to the green cheese to destroy bacterial action. Thus it seems that the chemical change caused in the non-protein portion of the milk by heating reduces the proteolytic action of rennet and of bacteria on cheese curd. H.A.B.

167. Discoloration and Corrosion in Canned Cream. C. J. JACOBSON, G. R. HOWAT AND T. P. HOAR, The Hannah Dairy Research Inst., Kirkhill, Ayr, and the Metallurgical Laboratories, Univ. of Cambridge. J. Dairy Research *8*, p. 285, Sept., 1936.

Two defects in canned cream were studied, namely, bronzing or purpling of the can and pitting of the cream with large areas of discolored cream at the site of the pitting and loose black specks scattered throughout the cream. The purpling was found to be due to a film of tin sulphide and the black specks to ferrous sulphide, stannous oxide or both. Cysteine and methionine are considered the most probable forms of "volatile" sulphur in canned cream, being produced by the effect of heat with consequent denaturation of the proteins.

Purpling was produced by excessive time and temperature of sterilization, appearing in this experiment after 20 hours of storage in cream sterilized at 117–118° F. for 40 minutes, but not at 117–118° F. for 30 minutes, and not appearing after six weeks of storage when the cream was sterilized at 114–115° F. for 40 minutes. Addition of sodium bicarbonate up to 5 grams per gallon had, if anything, a slightly beneficial effect. Homogenization pressure appeared to have no definite influence on the subsequent attack of the can.

It is possible that sodium bicarbonate somewhat increases the production of the slight pitting of the can. Cans in which steel had been exposed by filing gave on processing a black film at the exposed steel and later black specks throughout the cream.

Large air spaces in a can did not enhance attack on the cans nor did a variation of pH of the cream within the limits studied appear to have any particular effect. H.A.B.

Other abstracts of interest are numbers 168, 184, 188, and 189.

CONCENTRATED AND DRY MILK

Abstracts of interest are numbers 156, 158, 168, 172, 175, 179, 181, 184, 188, 189, 191, and 194.

CHEMISTRY

168. The Acidity of Milk and Dairy Products. H. H. Sommer, Univ. of Wisconsin, Madison, Wis. Wis. Agr. Exp. Sta. Research Bul. 127, Jan., 1935.

This bulletin is a discussion of existing knowledge of acidity in dairy products written for the benefit of dairymen and dairy technologists. It discusses the significance of pH; the acidity of freshly drawn milk; the constituents responsible for acidity of fresh milk; factors causing variations in acidity such as:—lactation, feed and mastitis; the acids formed by fermentation and the limitations of the acid test as a means of grading milk. There are careful discussions of the methods and significance of measurements of acidity in cream, condensed milk products and ice cream mixes. Scientific explanations are given for the value of pH measurements; effects of dilution of samples; and the fading of endpoint in titrations. A list of 27 references is given. W.V.P.

169. Ultracentrifugal and Electrophoretic Studies on the Milk Proteins. I. Introduction and Preliminary Results with Fractions from Skimmilk. Kai Oluf Pederson, Inst. of Physical Chemistry, The University, Upsala, Sweden. Biochem. J. *30*, p. 948, 1936.

In this report the author has extended the work of Svedberg and his associates in characterization of the proteins of milk by use of increased centrifugal fields wherein the force is up to 260,000 times that of gravity. A description is given of a modified method for calculating sedimentation equilibrium concentration from the refractive index method.

The caseinogen is shown to be present in course polydisperse suspension. When the caseinogen was removed by ultracentrifuging, practically the whole whey protein was found to be present as molecules α, β and γ, when α is a low-molecular protein isolated by Kedwick (unpublished), β the lactoglobulin isolated by Palmer, and γ corresponds probably to what is generally called lactoglobulin.

The results of these experiments indicate that it makes little or no difference whether the caseinogen is removed by isoelectric precipitation or by ultracentrifuging. K.G.W.

170. Ultracentrifugal and Electrophoretic Studies on the Milk Proteins. II. The Lactoglobulin of Palmer. KAI OLUF PEDERSON, Inst. of Physical Chemistry, The University, Upsala, Sweden. Biochem. J. *30*, p. 961, 1936.

In this paper the author reports the results of an ultracentrifugal study on lactoglobulin prepared according to the procedure of Palmer of the Carlsberg Laboratory. The lactoglobulin prepared from cows' milk was found to be a monodisperse protein with a molecular weight of 39,000 and an isoelectric point of 5.19 in acetate buffers. The molecular weight appears to be independent of hydrogen ion concentration from very acid values (pH 1.0) to pH 9. Above pH 11.0 light absorption increases strongly and the sedimentation constant decreases, indicating profound changes in the molecule, probably hydrolysis. While the molecular weight was found constant between pH 1.0 and 9.0, a change in sedimentation constant between pH 7.5 is believed due to change of the molecular frictional constant. The latter may be due to either change in shape or kinetic volume of the particle, as for example, due to change in hydration. K.G.W.

171. The Occurrence and Possible Significance of Some of the Minor Component Acids of Cow Milk Fat. THOMAS PERCY HILDITCH AND HARRY PAUL, Dept. of Industrial Chemistry, Univ. of Liverpool, England. Biochem. J. *30*, p. 1905, 1936.

Previous studies concerning the composition of milk fat have been extended to ascertain the validity of quantitative data of the major component acids, and to ascertain whether any unsaturated acid of lower molecular weight than decenoic acid is present, and also to obtain evidence as to the position of the unsaturated group in tetra and hexadecenoic acids the presence of which was indicated by previous experiments.

By comparison of calculations of analyses of the fat with those obtained when the fat was hydrogenated the presence of lower unsaturated fatty acids was indicated. In addition to decenoic and tetradecenoic acids, the presence of about 4–5 per cent hexadecenoic acids was determined. When allowance for the lower unsaturated fatty acids is made, a correction for the major acids equal to an increase of 2 per cent for palmitic and a decrease of 5 per cent for oleic is necessary. The positions of the double bond, relative to the carboxyl group are the same in the decenoic, tetra and hexadecenoic and oleic acids. It is suggested that these observations are in harmony with the hypothesis, that lower saturated glycerides of milk fats have been produced from pre-formed oleo-glycerides and that minor lower unsaturated components may represent degradation produces of oleo-glycerides which have escaped complete saturation to lower saturated groups. K.G.W.

FOOD VALUE

172. The Nutritive Value of Raw and Pasteurized Milk for Calves. The Assimilation and Retention of Nitrogen, Phosphorus and Calcium. JANET H. BLACKWOOD, SAMUEL MORRIS AND NORMAN C. WRIGHT, The Hannah Dairy Research Inst., Kirkhill, Ayr. J. Dairy Research *8*, p. 228, Sept., 1936.

Ten calves were fed during three 24-day-periods alternately on raw milk and milk pasteurized at 63–65° C. for 30 minutes and on another series three calves were fed raw milk and three pasteurized milk for 42 days. All calves were fed on dam's milk for three days and then raw milk up to 10 days of age.

The two types of milk produced no significant differences in the percentage assimilation and retention of nitrogen, phosphorus and calcium. Retention values varied markedly between individual calves. The mean retention values were highest for calcium, next highest for phosphorus and lowest for nitrogen.

It is concluded also that when milk is fed as a sole food for calves, it is relatively deficient in calcium and to a lesser extent in phosphorus. A calf when fed, as in these experiments, on milk at the rate of 10 per cent of its live weight will be unable to obtain adequate calcium, if the daily gain in live weight exceeds $\frac{1}{2}$ kg. With a live weight increase of $\frac{1}{2}$ kg per day, the phosphorus supplied will be inadequate and the calcium insufficient by over 40 per cent and with an increase in live weight of $\frac{3}{4}$ kg. per day, all three elements will be inadequate. The milk used contained 0.45 per cent nitrogen, 0.09 per cent phosphorus and 0.10 per cent calcium. The lack in calcium can be to some extent rectified by including pasture grass or hay in the ration. H.A.B.

173. Further Observations on the Effect of Dietary Caseinogen in the Prevention of Fatty Livers. ALAN WILMOT BEESTON, HAROLD

JOHN CHANNON, JOHN VAUGHAN LOACH AND HARRY WILKINSON, Dept. of Biochem., The University of Liverpool, England. Biochem. J. *30*, p. 1040, 1936.

Choline is known to have the property of preventing the accumulation, and accelerating the removal, of fat in the liver of the rat. It has been observed in experiments that the presence of protein in the diet had a similar effect and which is believed to act independently of contaminating traces of choline.

In continuance of these studies, the action of caseinogen has been equated against that of choline. One gram of caseinogen is equivalent in its preventative action on liver fat deposition to 7–8 mg. of choline. K.G.W.

174. Additional Observations on the Anemia Caused by Deaminized Casein. A. G. HOGAN, R. E. GUERRANT AND W. S. RITCHIE, Dept. of Agr. Chem., Univ. of Missouri, Columbia, Mo. J. Biol. Chem. *115*, p. 659, 1936.

Deaminized casein has been found to be inadequate as a nutritive protein source, and further, that it may cause anemia in animals when fed. The results of this paper show further data on the nature of the anemia producing property of the deaminized casein. The minimum amount of casein necessary to prevent anemia in experimental animals was 5 per cent of the ration; the minimum amount of deaminized casein required to produce anemia was found to be between 5 and 10 per cent of the ration. Lactalbumin of laboratory preparation does not prevent the type of anemia studied. Dried yeast 18 per cent confers protection against anemia caused by deaminized casein, while autoclaved casein or yeast does not. The active anemia producing agent was recovered in the sulfuric acid hydrolysate of deaminized casein, and in a similar manner, the anti-anemic agent was recovered from the hydrolysate of casein. K.G.W.

ICE CREAM

175. Escherichia-Aerobacter Group in Ice Cream. Proc. 36th Ann. Conv. Intern. Assoc. of Ice Cream Mfgrs. *2*, p. 7, Oct., 1936.

(A) Determination from a Laboratory Viewpoint. R. P. MYERS AND C. W. SORENSEN, Sealtest System Laboratories, Inc., Baltimore, Maryland.

In some laboratories considerable importance is attached to the presence in ice cream of organisms of the colon group. The test is occasionally used as a measure of the effectiveness of pasteurization. Since organisms within this group vary in thermal resistance and since pasteurization temperatures vary widely, it seemed desirable to establish the thermal resistance in ice cream for especially resistant strains of organisms within the colon group.

Two different strains of *E. coli* and one of *Aerobacter aerogenes* were selected for the study.

One strain of *E. coli* survived for a period of 20 minutes at 140° F. and for 5 minutes at 145° F. The strain of *A. aerogenes* survived for 10 minutes at 140° F. and for 5 minutes at 145° F. Neither organism would therefore survive pasteurization at 140° F. for 30 minutes. However, the second strain of *E. coli* was much more heat resistant. It survived for 118 minutes at 140° F., for 55 minutes at 145° F., for 24 minutes at 150° F., but for only 9.5 minutes at 155° F. On the basis of these results a pasteurization temperature of 155° F. for 30 minutes is recommended to insure complete destruction of colon group bacteria in ice cream mix.

Aging ice cream mix at 45° F. for 24 hours had no appreciable effect on the thermal resistance of *E. coli*.

DISCUSSION, LED BY E. H. PARFITT, PURDUE UNIVERSITY, LAFAYETTE, IND.

In studies at Purdue University, 67 cultures of *Escherichia coli* and *Aerobacter aerogenes* were selected and pasteurized in ice cream mix in capillary tubes. No colon type organisms were found which survived 145° F. for 30 minutes. Stark and Patterson, who isolated 505 pure cultures of Escherichia-Aerogenes organisms from water, also found that all the organisms isolated were destroyed at 145° F. for 30 minutes.

The paper of Myers and Sorensen shows clearly the need for uniform regulations as to time and temperature of pasteurization of ice cream mix. If the mix is pasteurized at 155° F. for 30 minutes and colon organisms are found in the finished product, a more accurate interpretation can now be placed upon these results than was possible previously.

(B) Its Significance and Importance. M. W. YALE, N. Y. State Agric. Exp. Sta., Geneva, N. Y.

The quality of bulk vanilla ice cream sold in Geneva was studied by the author. Through line run tests the sources of contamination were determined for a number of ice creams. The colon count proved to be a fairer measure of quality than the standard plate count since the freshly pasteurized mix in practically all cases was free from colon organisms. Thus, the presence of colon organisms is primarily an index to handling of the product subsequent to pasteurization. Discriminating manufacturers should include tests for colon organisms in finished products since their presence is always undesirable and indicative of some faulty condition.

Relatively high colon counts in the case of ice cream with low standard plate counts are significant and line run samples will usually show contamination either from ingredients or from equipment.

The colon plate count is more sensitive in detecting contamination from the vendor's dipper than is the standard plate count.

Since there are instances where total counts indicate faulty conditions but colon counts do not, a test for the colon group should supplement rather than supplant the standard plate count.

DISCUSSION BY A. C. FAY, KANSAS AGRIC. EXP. STA., MANHATTAN, KANSAS

The colon count has some value as an index to pasteurization especially if high temperatures are employed. If the usual thermal exposure of 145° F. for 30 minutes is employed the interpretation of the test must be made with caution. The test has some value as an index to the contamination of ice cream subsequent to pasteurization, especially if the freshly pasteurized product is known to be free from colon types.

The colon test serves as an index to the general sanitary conditions surrounding the production of ice cream, but in this capacity is a poor substitute for the total count. Since the standards of interpretation for colon counts are much lower than for total counts, the method affords an excellent means of demonstrating contamination of small magnitude such as from the vendor's dipper. Its value in this connection depends on the assumption that the contamination involves organisms of the Escherichia-Aerobacter group.

These limitations of the colon count are pointed out so as to enhance its value by avoiding the pitfalls of misinterpretation. Since any method of analysis has its limitations, intelligent interpretation of the results secured is essential if the greatest good is to result.

(C) At the Fountain. F. W. FABIAN AND A. E. HOOK, Michigan State College, East Lansing, Mich.

A sanitary survey was made of the 96 places in Lansing and East Lansing serving ice cream to the public. A bacteriological study was made of the water found in the receptacles holding the ice cream dippers. One city has a ruling that the dippers be kept in running water between servings while the other city has no such requirement.

At fountains where the dippers were kept in running water, the total bacterial count as well as the Escherichia-Aerobacter count of the water was less than where still water was used. Eighty-one per cent of the samples of still water had a plate count in excess of 100,000 bacteria per cc. while only 18 per cent of the samples of running water exceeded 100,000 bacteria per cc. Ten of the 18 per cent of samples of running water which exceeded 100,000 cc. were probably high because the water was running too slowly through the receptacle containing the dippers.

There is a general relationship between the total bacterial count and the Escherichia-Aerobacter content of retail ice cream when the count exceeds 100,000 bacteria per cc. Below this, the relationship is less marked. However, there seems to be no direct correlation between the Escherichia-Aerobacter content of the ice cream being served and the dipper water.

A correlation was observed between the general sanitation of the retail establishment and the bacterial content of the dipper water. Restaurants have a higher sanitary rating than pharmacies when the basis of comparison is the bacterial content of the dipper water. M.J.M.

176. The Relation of Fruit, Flavors, Nuts and Colors to the Sanitary Production of Ice Cream. Proc. 36th Ann. Conv. Intern. Assoc. of Ice Cream Mfgrs. *2*, p. 40, Oct., 1936.

(A) Importance of Problem from Public Health Point of View. M. J. PRUCHA, University of Illinois, Urbana, Illinois.

In the bacteriological examination of fruits, nuts, flavors and colors, extremely high bacterial counts were found in several dye solutions. Frozen fruits, flavors, nut meats and candy frequently contain several thousand bacteria per gram. Molds and yeasts are present in many samples of frozen fruits, color solutions and nut meats. *Escherichia coli* were found in some color solutions, candy and nut meats but not in frozen fruits or flavor extracts. However, there is no definite evidence that these materials had infected ice cream with disease bacteria and that such ice cream caused epidemics.

The proper inspection and control by health officers of plant operations where fruits, nuts, colors and flavors are manufactured and handled is recommended. It is also suggested that the ice cream manufacturer take proper care of these ingredients by the following methods:

1. Keep the ingredients under proper sanitary conditions.
2. Treat the ingredients so as to keep them safe.
3. Purchase only good products from companies exercising sanitary precautions.
4. Carefully pasteurize the mix and guard against recontamination of the product after pasteurization.

(B) Importance of Problem from Ice Cream Manufacturer's Point of View. H. F. DEPEW, Luick Ice Cream Co., Milwaukee, Wis.

Contamination by workers of flavoring materials and colors should be avoided so far as possible, particularly with fruits and nuts which are not pasteurized.

The recommendation is made that color solutions be made in quantities of about a week's supply and that they be kept refrigerated. The pasteurization of color solutions is recommended. Pasteurization of colors does not result in off-shades or fading of the color. The use of preservatives in color solution is not recommended.

Nut meats should be kept in a dry, cold storage at about 32° F. The purchase of fumigated nuts is probably desirable. All choppers, slicers, and other equipment coming in contact with nuts should be carefully cleaned and sterilized before use.

The opinion is expressed that the pasteurization of all fruits used in ice cream is inadvisable. A cooked or preserved flavor was evident in the resultant ice cream and the fine, fresh, clean fruit flavor was lost when the fruit was pasteurized. Fruit purchased from reputable packers was found to be reasonably low in bacterial content. Fruit should be stored at temperatures near 0° F. and should be thawed in a room having a temperature between 32 and 40° F. Only fruit for immediate use should be thawed.

Flavors and syrups should be pasteurized when practical. Only the best quality of raw materials should be used in their manufacture and all utensils and containers should be cleaned and sterilized before use.

Since specific sanitary procedures have not been developed for handling fruits, nuts, flavors and colors, the industry is urged to study the problem so as to learn the practicability of various proposals as well as future proposed legislation along this line. M.J.M.

177. A Study of Methods Used to Improve the Sanitary Quality of Nut Meats, Flavoring and Coloring Used in Ice Cream. P. H. TRACY AND W. H. BROWN, Univ. of Ill., Urbana, Ill. Proc. 36th Ann. Conv. Intern. Assoc. Ice Cream Mfgrs. *2*, p. 52, Oct., 1936.

Different methods of soaking nuts previous to shelling were studied and were found to increase the moisture content of the resultant nut meats. The soaking process injured the texture of the nuts.

Since nut meats often show evidence of human contamination, methods were studied for improving the sanitary quality of nuts. The best treatment studied was to dip the nuts into hot (180° F.) sucrose solution for 30 to 60 seconds, then dry for 2.5 minutes in a 250° F. oven. The nuts should then be kept in cold storage, preferably in Glassine bags, until used. Nuts treated in this way contained less than 100 bacteria per gram and were free from *Escherichia coli*.

Heating frozen pack strawberries previous to use is recommended as a means of lowering the bacterial content of this ingredient. Pasteurization of the berries at 145° F. for 30 minutes was practically as efficient as higher temperatures and the flavor was injured but slightly at the low temperature.

Experiments with flavoring extracts pasteurized at 145° F. for 30 minutes indicate that, in case there is doubt as to the sanitary quality of the extract, heating can be resorted to without decreasing the value of the flavor for ice cream. The trials indicate that vanilla extract subjected to this treatment can be used satisfactorily.

Pasteurization of color solutions proved successful. There was no injury to the quality of the color providing the temperature did not exceed 145° F.–160° F. M.J.M.

178. Frozen Cream—The Best Method of Handling and Use. R. R. GOCKLEY, Dairymen's League Cooperative Association, New York,

N. Y. Proc. 36th Ann. Conv. Intern. Assoc. Ice Cream Mfgrs. *2*, p. 68, Oct., 1936.

Since ice cream made entirely from frozen cream has a different flavor than that made from fresh cream, the use of frozen cream for more than 60 per cent of the butterfat is not recommended.

Frozen cream can be satisfactorily melted (1) by storing over night at room temperature until it is sufficiently melted to permit dumping from the cans or (2) by setting the cans in a tank of water controlled to a definite temperature. The average melting time by either method does not permit increases of significance in bacterial content of the cream. Some companies have utilized an ice crusher to crush the frozen cream. The maintenance of sanitary conditions is difficult with this method.

Clean flavored frozen cream has been reconstituted and sold as fluid cream without objections from the buyer. M.J.M.

179. Cooperation between Accounting and Production Departments for Increased Plant Efficiency. L. C. ANDERSON, General Ice Cream Corp., Schenectady, N. Y. Proc. 36th Ann. Conv. Intern. Assoc. Ice Cream Mfgrs. *2*, p. 70, Oct., 1936.

The accounting department should equip the production department with adequate inventory and factory record forms so that complete information as to mix making costs, freezing costs, etc., are properly arrived at. Cooperation between accountant and production man is necessary to produce reliable cost figures and the use of these data by the production man will aid in increased plant efficiency. M.J.M.

180. Sales Potentialities of Ice Cream. AUBYN CHINN, National Dairy Council, Chicago, Ill. Proc. 36th Ann. Conv. Intern. Assoc. Ice Cream Mfgrs. *1*, p. 73, Oct., 1936.

The use of ice cream twice a week in the home is suggested as a modest goal for which to strive. This offers a potential increase of 300 per cent in the use of ice cream over present consumption. The present popularity of ice cream outside the home indicates that the success of any program for increased sales is limited only by the effort with which the program is carried out.

The following suggestions are offered as methods for placing ice cream consumption on a permanently higher plane.

1. Place before the professional leaders in the medical and educational fields, the quality and superior food value of commercial ice cream.
2. Integrate projects and material on ice cream in the regular educational program in schools.
3. Increase consumer knowledge of the composition, and the manufacture of commercial ice cream using all publicity and educational channels, such as newspaper editorials, leaflets for direct mail to consumers and food demonstrations.

4. Stimulate increased use of ice cream for young adults in factories and offices for between-meal pick-up through appeals to beauty, weight control and physical finess. M.J.M.

181. Production Planning in Relation to Personnel. RIDGWAY KENNEDY, JR., Abbotts Dairies, Inc., Philadelphia, Pa. Proc. 36th Ann. Conv. Intern. Assoc. Ice Cream Mfgrs. *2*, p. 78, Oct., 1936.

The accountant should supply the production department with production schedules for the same month of previous years. The figures should be divided into the various units as bulk, package, brick, cups, novelties, etc. The production department should know the amounts of each product which can be turned out for each shift. From these figures the number of workers necessary can be ascertained.

Different departments of the business do not have peak loads at the same time. Therefore trained workers should be retained whenever possible and merely shifted from one department to another. Extra help should be employed prior to the peak load so that they will be familiar with the work by the time that maximum production must be obtained. M.J.M.

182. Organization and Operation of a Cabinet Service Department. O. F. BOYER, Ice Cream Refrigeration Service Co., Los Angeles, Calif. Proc. 36th Ann. Conv. Intern. Assoc. Ice Cream Mfgrs. *2*, p. 81, Oct., 1936.

Service department costs are entirely dependent upon the make, age and condition of refrigeration equipment as well as the amount of the dealer's equipment which the sales department agrees to refrigerate. Multiple hook-ups on a single compressor usually cause trouble. The nearer one stays to one cabinet on one compressor, the cheaper will be the refrigeration costs.

All cabinets should be inspected regularly at intervals of about four times yearly. Large cabinets are no more costly to service than small ones. A fair average cost per compressor, per month, for maintenance is about $1.50.

M.J.M.

183. Economical Layouts for the Manufacture of Novelties. JOHN W. BURDAN, Philadelphia Dairy Products Co., Philadelphia, Pa. Proc. 36th Ann. Conv. Intern. Assoc. Ice Cream Mfgrs. *2*, p. 87, Oct., 1936.

A description is given of a satisfactory layout for making Popsickles, Fudgicles, etc.

The author suggests that in planning a novelty department first consideration be given to the question of sanitation since these operations can easily become insanitary. Such problems as the economical handling of materials to prevent waste, increased efficiency of labor, refrigeration, etc., should be decided only along lines which will not conflict with sanitary arrangements.

M.J.M.

184. The New Developments at the Dairy Industries Exposition. BYRON MORRIS, Perry Creamery Co., Tuscaloosa, Alabama. Proc. 36th Ann. Conv. Intern. Assoc. Ice Cream Mfgrs. *2*, p. 92, Oct., 1936.

In this paper and the lengthy discussion following it, which also is recorded in the proceedings, many of the outstanding developments in machinery and methods which were shown at the 1936 Dairy Industries Exposition are discussed. M.J.M.

185. Flooded and Refrigerant Control System. FRED APHULS, Consulting Engineer, New York, N. Y. Ice and Refrig. *91*, 2, p. 146, Aug., 1936.

The author points out that the flooded system makes more effective use of the cooling system, permits smaller mean temperature differences, and facilitates efficient operation. It is pointed out that for efficient operation, the temperature of the vapor entering the refrigerating machine should be kept 10° F. higher than the boiling point of the liquid at the indicated suction pressure. Five methods for regulating the liquid flow to the refrigeration machine are briefly described and evaluated. A liquid separator located above the cooler may be used together with a liquid return line to the bottom of the cooler. A pump may be used for returning unevaporated liquid to the bottom of the cooler. A low pressure float valve may be used. A liquid trap in the suction main with a return pipe to the bottom of the cooler may likewise be used. Suction stop valves, thermostatically controlled may also be used. Emphasis is placed upon designating a system which gives the operator an opportunity to see what is going on. L.C.T.

186. Frozen Desserts. P. H. TRACY. Ill. Agr. Exp. Sta. Cir. 462, Nov., 1936.

"Nutritious, attractive, inexpensive, and convenient to serve, ice cream deserves the great popularity it has in the American menu." This circular discusses the ingredients of ice cream and describes the preparation of ice cream mixes for use in the home.

Various types of freezers and their use in the home are described.

Many recipes for home-made ice creams, ices and sherbets are given and also directions for preparing fancy deserts. O.R.O.

187. Ice Cream Sales Index. The 41st Production and Distribution Survey of the Statistical and Accounting Bureau, Intern. Assoc. of Ice Cream Mfgrs., Washington, D. C.

This survey of ice cream sales in the United States for the first nine months of the year 1936, when compared with the same months of the year 1935, shows an increase of 20.64 per cent in sales of ice cream. The increase in sales for the same period in the Dominion of Canada was 9.82 per cent.

The effect of the Kentucky sales tax of 28 cents per gallon for ice cream, which went into effect on July 1, 1936, is shown graphically in this survey. During the month of June (the last month before the tax became effective) sales of ice cream in Kentucky increased 59.39 per cent over the like month of the previous year. During the months of July and August (with the tax in force) ice cream sales decreased 14.17 and 15.43 per cent, respectively, when compared with the same months in 1935. In the states adjacent to Kentucky, increases in sales of 33.11 and 23.86 per cent, respectively, occurred in these same months. M.J.M.

Other abstracts of interest are numbers 157, 159, 161, 163, 168, 172, 188, 189, 191, and 194.

MILK

188. The Comparative Values of the Plate Count and the Modified Methylene Blue Reduction Test as Routine Methods for Grading Milk. A. A. NICHOLS AND S. J. EDWARDS, The Hannah Dairy Research Inst., Kirkhill, Ayr. J. Dairy Research *8*, p. 258, Sept., 1936.

In a large number of comparisons it was found that a remarkably high correlation exists between the results of the plate count and the methylene blue reduction test. Correlation coefficients were calculated by the extended method. Regression equations were determined to express one variable in terms of another. Plate counts were expressed as logarithmic values.

Both the plate count and the reduction test also show a fairly high correlation with the keeping quality and with the presumptive coliform test. The correlations with the laboratory post-pasteurization counts are only moderate.

Correlations between farm inspection scores and plate count or reduction test were of a low order. The farm score card allowed 150 points for methods of production and 50 points for equipment. A good correlation, however, was noted between the tests and the storage temperature of the milk. The storage temperature affects the reduction test to a greater degre than the plate count indicating that the reduction test is more sensitive to growth of bacteria than the plate count. For every increase of 4° F. in the temperature of storage there was on the average a decrease in reduction time equivalent to 70 minutes and an increase in the plate count of roughly 150 per cent.

A high count in mixed milk of the B haemolytic variety of *Streptococcus agalactiae*, most commonly responsible for mastitis in cows, was found to be associated with a high total plate count and a short reduction time. The presence in mixed milk of milk infected with streptococcal mastitis did, therefore, not invalidate the methylene blue reduction test.

It is concluded that the reduction test, although it involves an inherent error of about 17 per cent due to the half hour periods between readings, is

likely to give more consistent and more easily duplicated results than the plate count. H.A.B.

189. The National Dairy Council. Miss Aubyn Chinn, National Dairy Council, Chicago, Ill. Milk Dealer *26*, p. 30, Dec., 1936.

A complete description of the eight-point program of the National Dairy Council. The author also gives details as to how the council functions and a summary of what the council did last year. C.J.B.

190. Certified Milk—The Oldest Graded Milk in the World. Harris Moak, Milk Comm., Kings Co. Medical Society, Brooklyn, N. Y. Milk Dealer *26*, p. 42, Dec., 1936.

The author presents a brief history of Certified Milk.

C.J.B.

191. Recent Work of the Public Health Service on Milk Pasteurization. Leslie C. Frank, Office of Milk Investigations, U. S. Public Health Service, Washington, D. C. Milk Dealer *26*, p. 48, Dec., 1936.

A summary of the milk sanitation work of the U. S. Public Health Service.

C.J.B.

192. A Quarter of Century in Retrospect. H. H. Sommer, University of Wisconsin, Madison, Wis. Milk Dealer *26*, p. 60, Dec., 1936.

The author reviews the progress of the fluid milk industry during the past 25 years. The review covers new and improved types of equipment, methods of processing, enactment of ordinances, nutrition, etc.

C.J.B.

193. The Milk Marketing Program of the American Jersey Cattle Club. James D. Brew, American Jersey Cattle Club, New York. Milk Dealer *26*, p. 68, Dec., 1936.

The author briefly outlines the program of the American Jersey Cattle Club for the marketing of "Jersey Creamline Products." C.J.B.

194. New Developments in the Field of Vitamin D Milk. W. E. Krauss and R. M. Bethke, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Bi-monthly Bul. *22*, p. 3, Jan.–Feb., 1937.

This is a revision of a previous article (Ohio Agr. Exp. Sta. Bi-monthly Bul. 20, March–April, 1935) and brings up to date the status of vitamin D milks. Details of the various methods used to fortify milk with vitamin D are given, methods of control are discussed, clinical evidence on the value of vitamin D milks is brought up to date, data on the present output are given, and suggestions are made for maintaining a proper perspective in developing this phase of the dairy industry. W.E.K.

195. Legume Silage for Dairy Cows. C. C. HAYDEN, A. E. PERKINS, W. E. KRAUSS, C. F. MONROE AND R. G. WASHBURN. Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Bi-monthly Bul. *22*, p. 21, Jan.–Feb., 1937.

Three systems of ensiling legumes are discussed: (1) no treatment other than a brief drying period in the field; (2) addition of feeding molasses; (3) addition of mineral acids (A. I. V. system). Of these, the mineral acid method has received particular attention and has given uniformly best results with respect to carotene preservation.

Data presented show that the feeding of large quantities of A. I. V. silage over a period of five months exerted no detrimental effect on the cows, although ammonia in the urine markedly increased and bicarbonates markedly decreased. The CO_2 capacity of the blood serum was practically unchanged.

In a reversal feeding trial no difference was found between the production value of A. I. V. silage and alfalfa hay when fed on approximately the same dry matter basis.

Carotene was preserved to a high degree in the A. I. V. silage. This resulted in maintaining a high carotene content in the butterfat of both Holsteins and Jerseys throughout the winter when silage was the only roughage fed. In the reversal feeding trial the cows produced butterfat of much higher carotene content when receiving A. I. V. silage and corn silage than when receiving alfalfa hay and corn silage of equivalent dry matter content.

The question, "Is there a Place for Legume Silage?" is discussed with an affirmative trend. W.E.K.

196. Sampling Is Aid in Locating Milk Troubles. C. F. MONROE, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Weekly Press Bul. *22*, p. 47, Jan.–Feb., 1937.

In order to help locate the source of off-flavor or other abnormalities in milk it is suggested that samples be drawn from individual cows into sterile containers. After allowing to stand at room temperature for several hours, examination for off-flavor and physical defects will either establish the cow as the offender or suggest that the trouble lies somewhere in the process of handling the milk after it leaves the cow. W.E.K.

197. Are Your Sales Receipts Up to Delivery Costs? WAYNE DINSMORE, Secretary, Horse and Mule Association of America. Milk Dealer *26*, p. 66, Dec., 1936.

The single horse and wagon is the milkman's ideal delivery unit because it delivers milk for an average of less than 5 per cent of sales anywhere, East, West or South. The average motor truck route cost of sales ranges from 7 per cent to 10 per cent of sales. No single horse route at present

should exceed $50 per month in total cost—horse, wagon, harness and stable overhead, all included. The average, companies from 29 states included, is $46.86. The average motor truck cost from companies in the same 29 states is $80.78. The average horse route sells more milk than the average motor truck route. The horse is the business getter, the salesman's helper, who handles the wagon while the salesman takes care of the milk.

The author presents cost figures to substantiate the above statements.

C.J.B.

Other abstracts of interest are numbers 156, 158, 168, 172, 175, 178, 179, 181, 184, and 185.

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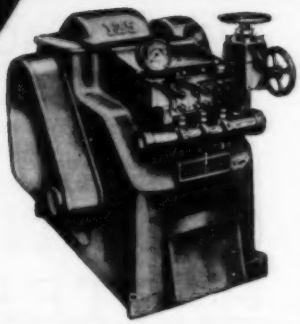

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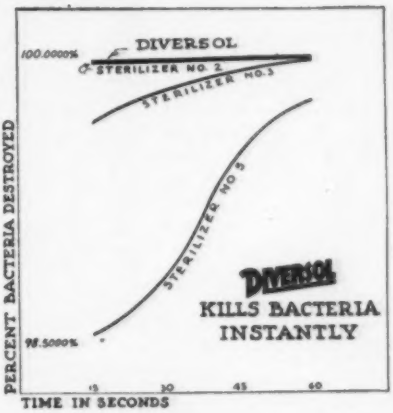

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